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GELATINIZATION OF LOW MOISTURE WHEAT STARCH

by

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INTRODUCTION

Starch is a very important biopolymer and it has numerous applications even beyond the food industry (Dengate 1984). What makes starch such a useful entity is its ability to undergo a drastic change when heated in the presence of water. This change is referred to as gelatinization. Native starch and gelatinized starch provide much of the texture, mouth feel, and structure for many of the foods we eat (Harper 1981). Starch has been extensively studied because of its prominent position in the food industry - yet surprisingly little is actually known about gelatinization at the molecular level. A more thorough understanding of this phenomenon would assist in the study of such starch-based products as breads, crackers, cakes, and cookies. What is now known or speculated about gelatinization, however, can be used to guide current research in appropriate directions. A complete review covering all aspects of starch and gelatinization can be found in the book edited by Whistler et al. (1984).

GELATINIZATION

The granular structure of starch is important to consider when discussing gelatinization. As previously mentioned, many good reviews of this topic exist in the literature; a brief overview will provide the necessary background to establish the

relationship between structure and gelatinization. The starch granule is organized in a ringed structure, analogous to that found with onions, that may reflect the day-to-day deposition of starch in the developing granule. Amylopectin molecules are oriented perpendicular to these growth rings (also to the outer surface of the granule); that is, they are aligned along an imaginary axis extending from the center of the granule, the hilum, radially towards the edge of the granule. Portions of these long, branched molecules are arranged in orderly formations, the crystallites, which are also perpendicular to the rings (French 1984). It is the regular orientation of the amorphous and crystalline regions that give the granule its characteristic birefringent pattern, known as the Maltese Cross, when viewed with crossed polarizers in a light microscope (Zobel 1984). The noncrystalline, or amorphous regions, contain the amylose molecules and sections of amylopectin molecules not involved in crystallites (Banks and Greenwood 1975). Absorbed water will hydrate the amorphous areas, and is able to move rather freely within this portion of the granule.

When wheat starch is placed in cold water, the granules will absorb the solvent until the total water content is approximately 30% (w.b.). This corresponds to an increase in the diameter of the granule of about 20%; this process is also reversible (Dengate et al. 1978). When heat is applied to such a system, however, permanent changes begin to occur. At the initial gelatinization temperature, the granules begin to swell rapidly and lose their birefringence which indicates that the ordered regions are being disrupted. These changes start at the hilum

and spread quickly to the periphery of the granule (Olkku and Rha 1978). An individual granule gelatinizes over a narrow temperature range, 0.5°-1.5°C per granule, and a population of starch granules will melt over an approximate range of 10°C (Gough and Pybus 1971). In addition to the loss of crystalline order, gelatinization is characterized by a significant uptake of water, resulting in a tremendous expansion of the granule, and the leaching of amylose molecules into solution (D'Appolonia et al. 1978).

The process described above is for starch that is heated in an excess of water. When the total water in the system is less than 66%, gelatinization will involve more than one mechanism (Donovan 1979). A good way to illustrate the differences between low moisture and excess water gelatinization is with the differential scanning calorimeter. Typical thermograms for water-to-starch ratios of 2:1, 1:1, and 0.5:1 are provided in Figure 1. Burt and Russell (1983) have labeled the endotherms G (the excess water gelatinization peak), M_1 (the higher temperature crystalline melting endotherm characteristic of low moisture starch samples), and M_2 (the amylose-lipid complex melting peak). Although the result is the same, disruption or melting of the crystallites, the low temperature endotherm, G, and the higher temperature endotherm, M_1, are most likely the result of different mechanisms. As can be seen in Figure 1, a small G endotherm is still present even in the 0.5:1 sample (33% moisture), presumably due to the presence of just enough water to cause some crystallite disruption by the excess water mechanism. When water becomes limiting, the crystallites will be disordered

by a melting phenomenon in which individual granules will now be disrupted over a wider temperature range (Donovan 1979).

GELATINIZATION MECHANISMS

The paper generally considered to be the first exhaustive attempt at understanding and explaining gelatinization at the molecular level is credited to John Donovan (1979) in his classic Biopolymers article. In the paper, he used a differential scanning calorimeter (DSC) to study potato starch and formulated hypotheses on the mechanism of starch gelatinization in excess amounts of water and also in limited water situations. Donovan believed that the same type of order-disorder transiton occurs independent of moisture content, but that excess water gelatinization proceeds by a mechanism different from pure crystalline melting. For the low temperature endotherm associated with disordering the granule at high moisture contents, he attributes the transition to a "stripping" of chains from the crystallites due to the swelling of the amorphous regions of the granule by imbibed water. When the amount of water in a starch sample is below 66%, crystallites located in areas where the water concentration is high enough would undergo this stripping process, while the remaining crystallites simply will melt, thus producing two endothermic peaks.

This article was very important in initiating most of the subsequent research for a mechanism of starch gelatinization. Careful examination of his hypotheses has raised a few questions. For example, "stripping" can adequately account for the narrow temperature range over which individual granules will gelatinize.

Swelling of the granule during gelatinization occurs rapidly in excess water; this could explain why all the crystallites in a granule will melt quickly, but will be valid only if swelling is the cause and not an effect of gelatinization. On the other hand, the stripping mechanism cannot satisfy the observation that gelatinization is seen to begin in the center of the granule and proceeds radially. Although swelling is thought to begin at the center of the granule (Olkku and Rha 1978), Donovan makes no provision for this as "stripping" is presented as a randomly generated phenomenon.

Another mechanism for excess water gelatinization, one quite similar to that of Donovan's, is offered by Biliaderis et al. (1980). They assert that the hydration and swelling experienced by the amorphous regions when the granule is placed in water make the crystallites more susceptible to melting when the sample is heated, allowing the granule to be disordered quickly. More specifically, water may assist in the melting of the crystallites by exerting a solvation assisted helix - coil transition of the chains of the crystallite molecules. As water becomes limiting, melting by this process decreases, and the extent of solvation assisted melting now depends on the amount of water that will be redistributed around the crystallites. With less and less water available, the system approaches a true crystalline melting phenomenon. This mechanism has basically the same shortcomings as the previous one. It can adequately account for the narrow temperature range over which each individual granule will melt, but the excess water disordering process is not organized, i.e., the solvent assisted melting phenomenon is not defined as moving

from inside the granule to the outer edge. As the hypothesis is presented in the article, it is assumed that it does not matter where the first crystallites begin melting within the granule - yet gelatinization progresses in a very definite pattern.

Evans and Haisman (1982), working with potato starch, have suggested another possible mechanism for gelatinization. The process they outlined is dependent on the stability of the crystallites. Each granule will contain crystallites that have a range of stabilities - those which contain the least stable crystallites in the sample will begin to change first with the application of heat. These granules can now absorb slightly more water since they are not held as rigidly in conformation. The excess water would lower the stability of nearby crystallites, which will melt, allowing the granule to absorb even more water. This again lowers the stability of more crystallites which will melt, and so on. Hence, each granule will be disordered fairly rapidly because of this highly cooperative process. Thus, the temperature at which each granule will be gelatinized is determined by the melting point of its least stable crystallite. The range of stabilities will have no effect on the amount of time it takes for an individual granule to gelatinize because the process is so cooperative, but the gelatinization temperature range of the population of granules will be determined by the distribution of least stable crystallites. When the water content of the sample is decreased, granules will gelatinize according to this mechanism until there is no longer any free water available, at which time crystalline melting takes over.

Once again, this mechanism has some deficiencies. This

hypothesis is the most plausible in explaining the sharpness with which each individual granule will be disrupted in excess water. However, it also fails to account for the observation that gelatinization begins in the center of the granule and proceeds radially. Considering all three hypotheses, this mechanism is probably the most random - gelatinization will start where the least stable crystallite is located in the granule. Once gelatinization would begin, it would seem likely that incoming water would make crystallites towards the outer edges of the granule less stable first and gelatinization would be most likely to occur from the outer edge towards the hilum. This is just the opposite of how it actually occurs. In addition, this hypothesis would predict that if starch could be made to absorb more water before gelatinization, the granules would be disrupted at a lower temperature and over a narrower temperature range because of an increase in the destabilizing effect of water when the first crystallites begin to melt. Naegeli amylodextrins, produced by digesting the amorphous areas in the granules with sulfuric acid, are granules that will absorb more water than native starch. When Naegeli amylodextrins are heated, crystallite disruption does begin at a lower temperature. However, instead of gelatinizing quickly, the crystallites in these granules melt over a rather broad range of temperature, even wider than that for native starch. Therefore, this model, like the others discussed here, is not totally adequate.

GLASS TRANSITION

Recently, Maurice et al. (1983) have proposed that the

amorphous regions of starch granules should exhibit a moisture dependent glass transition. This second order transition is common to most polymeric substances - it is characterized by the transformation of the amorphous regions from a glassy state to a rubbery state with the application of heat (Brydson 1972). No direct evidence exists that such an event occurs in starch, but it is noted that gelatinized samples exhibit a DSC baseline shift indicative of the change in heat capacity associated with polymers that experience a glass transition (Billingham and Jenkins 1972).

If starch does exhibit a glass transition, it would help to explain some of the gaps in the understanding of gelatinization. For instance, despite the fact that the exact role of the amorphous areas in gelatinization is not known, the behavior of Naegeli amylodextrins makes it obvious that the noncrystalline regions are very important to the excess water gelatinization process. Since a glass transition will affect the amorphous areas, it is quite possible that the influence these regions have on gelatinization is, in part, due to this second-order transition. In addition, since water acts as a plasticizer for the starch molecules, the glass transition temperature will be dependent upon the water content of the sample. Thus, the melting point of the crystallites will also be dependent on the water content of the sample. This is a good explanation for the observed upward shift in melting temperature with decreased amounts of water in a sample.

ANNEALING

Another polymeric phenomenon exhibited by starch is annealing of the crystallites. This was first reported by Gough and Pybus (1971) who treated wheat starch in 50°C water for 72 hours, producing a sample that possessed an increased gelatinization temperature and a narrower temperature range for the transition. The same results were reported by Marchant and Blanshard (1980). According to French (1984), slow rates of heating facilitate the annealing process which may include realignment of the starch chains in the amorphous areas and some additional crystallization. This would result in an increase in the gelatinization enthalpy as seen with the DSC; however, this view would be questioned as some believe the smaller, less perfect crystallites may melt and more perfect crystallites form, causing no real increase in ΔH (Banks and Greenwood 1975).

CONCLUSIONS FROM THE LITERATURE

As was discussed earlier, none of the proposed mechanisms for starch gelatinization found in the literature are totally adequate. A more recent model for excess water gelatinization has been suggested by Hoseney (1984). Unlike those mentioned previously, this mechanism can account for all aspects characteristic of the order-disorder transition. Earlier, it was stated that starch granules have a radially disposed ring substructure. It would seem natural for the crystallites between the hilum and the first growth ring (if they exist) or the crystallites between the first and second growth rings to be under more stress than those further from the center of the

granule. Crystallites grow circumferentially, or at right angles to the orientation of the amylopectin molecules (French 1984). Because the rings closest to the center of the granule will have a very small radius of curvature, it will be more difficult for the amylopectin chains to be oriented in such a manner to allow the formation of crystallites. In other words, amylopectin molecules that are associated with crystallites in these innermost rings will have more of a fan-like projection, whereas molecules associated with crystallites in the outer growth rings can remain relatively parallel to one another as they are laid down along the circumference of the developing granule - a more stable conformation.

The crystallites under the most stress should be the most likely to melt first; this is apparently the case since gelatinization, as observed with a light microscope, proceeds from the center towards the surface of the granule. Once these crystallites are disordered, hydration of the newly exposed hydroxyl groups will occur. Since the starch is in an excess of water, solvent is thoroughly dispersed throughout the granule's amorphous areas. A flow pattern will thus be established bringing water from outside to inside the granule due to the movement of water towards the hilum to accomodate the changes taking place there. The increased swelling at the center of the granule caused by the additional water will be the driving force to stress the next growth ring. As new crystallites are melted, more hydroxyl groups are exposed causing more water to flow into this area of the granule. The swelling is further increased, additional rings are stressed, and so the process continues until

the entire granule is disordered, and fairly rapidly, by this "snowball" or cooperative effect. When water becomes limiting - no excess water remains to be pulled inside the granule - a crystalline melting phenomenon now occurs. If amylopectin molecules span more than one growth ring (French 1984), additional stress may be present since some crystallites would contain amylopectin with mobile ends. Finally, whether or not starch granules experience a pressure increase during gelatinization still appears to be an open question (Sterling 1978).

OBJECTIVE

Many starch-based foods are prepared from a limited water system, i.e., breads, crackers, and cookies. The extent to which the starch granules are altered by the cooking or baking treatment will have a tremendous effect upon the textural properties of the product. Thus, a thorough understanding of gelatinization becomes increasingly important in order to study these starch/water systems. Unfortunately, relatively little is known about this order-disorder transition except what can be seen or measured on a macroscopic scale. Hence, it was desired to study starch gelatinization at the molecular level with the hope of applying this knowledge to the thermal treatment of starchy food products.

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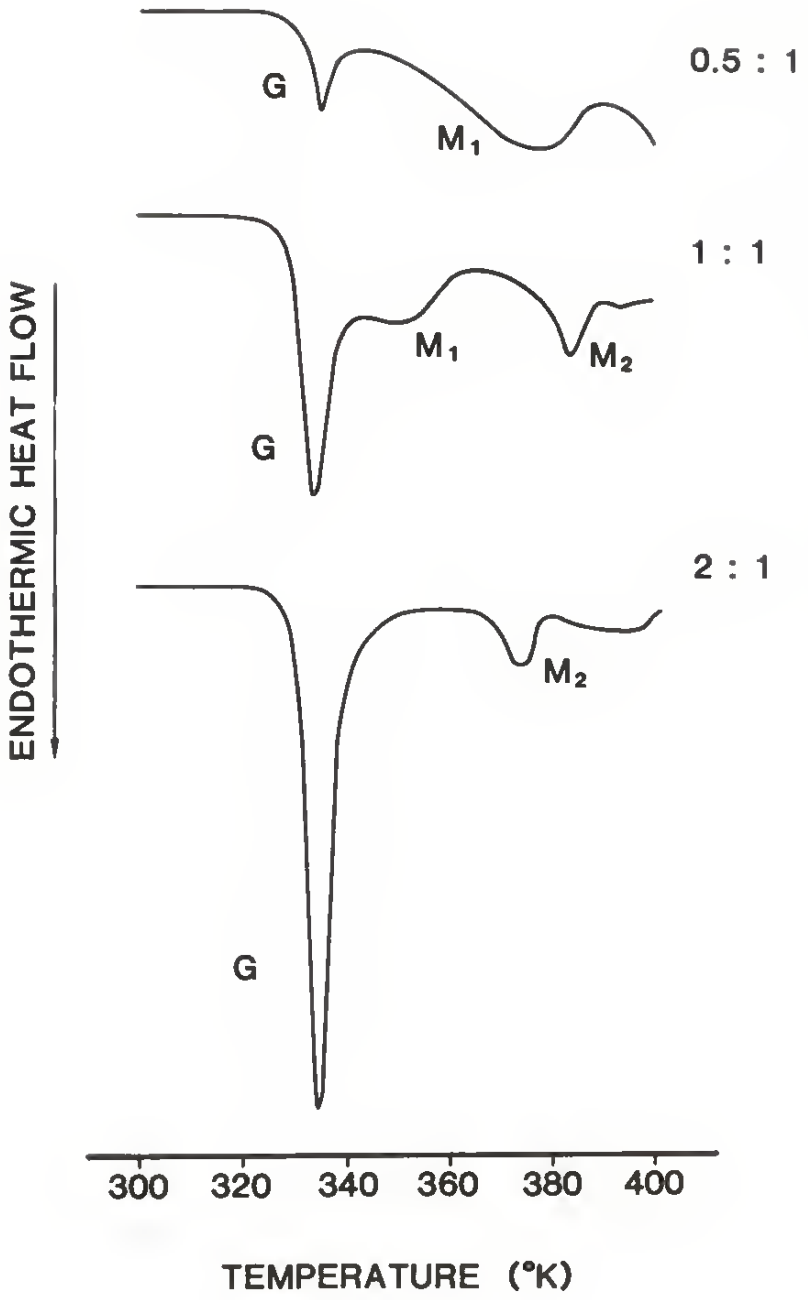
Figure 1. DSC thermograms of wheat starch at water-to-starch ratios of 2:1, 1:1, and 0.5:1. Moisture contents (wet basis) are 66%, 50%, and 33%, respectively. A heating rate of 10°K per minute, a chart speed of 10 mm per minute, and a sensitivity of 0.5 mcal per second was used for all samples.


0.5 : 1
G
M1
1 : 1
M1
M2
G
2 : 1
M2
G
ENDOTHERMIC HEAT FLOW
300
320
340
360
380
400
TEMPERATURE (°K)

CHAPTER 2

ADIABATIC CALORIMETRY

INTRODUCTION

One of the main objectives of this project was to gain a better understanding of the gelatinization phenomenon of starch, and to apply this knowledge to the thermal treatment of low moisture systems, such as food extrusion. Gelatinization, at the molecular level, is still not thoroughly understood. Of all the hypotheses for gelatinization mechanisms, one hypothesis for an excess water mechanism which seemed reasonable and warranted further investigation was the proposal by Donovan (1979), which predicted a "stripping action" on the starch crystallites during heating in the presence of water. In addition, he suggested that gelatinization enthalpy consists not only of the energy required to melt the crystallites, but also of the energy associated with granule swelling, denaturation, and the hydration of starch molecules as well. These latter three events are distinctly separate from melting, and have been grouped together and referred to as 'mixing' within the granules during gelatinization.

This proposed mixing phenomenon became a very important concept to help explain the notion that the enthalpy of gelatinization (ΔH) is dependent upon the water content of a starch sample. It is still widely accepted that as the amount of water in a sample decreases, the energy associated with the melting endotherm (the second endotherm or higher temperature

endotherm) also decreases (Donovan 1979 and Eliasson 1980). Thermodynamically, enthalpy is a state function; therefore, the ΔH associated with gelatinization should be constant if melting of the crystallites is the only event occurring (Atkins 1978). If the enthalpy does indeed decrease, then it seems likely that more than one process is involved. Thus, 'mixing' can account for the decrease in enthalpy - as less water is present in the system, swelling (endothermic), denaturation (endothermic), and hydration (exothermic) also decline and the system approaches a true melting phenomenon.

If mixing cannot account for the decline in ΔH, then it would seem likely that not all of the starch crystallites have been melted after heating a low moisture sample through its melting endotherm (since the enthalpy of this peak will decline), and that these remaining crystallites could possibly be disordered under different conditions. Donovan (1979) reheated a low moisture starch sample and observed no thermal event. He then heated a new sample (no moisture content was specified, but the melting endotherm occurred in its entirety within the DSC scan), cooled it, opened the pan and transferred this treated starch to another pan with an excess of water, expecting to see a small, excess water gelatinization response. When this pan was run in the DSC, again, no thermal event occurred, leading him to conclude, "It thus appears that in the presence of small amounts of water, if ordered portions of the starch sample remain after heating through the temperature range of the endotherm, the addition of water at room temperature is sufficient to disorder them." This effect - the disordering upon the addition of water

- would constitute 'mixing', but it could not be seen with a scanning calorimeter because the addition of water must take place outside the instrument. An adiabatic calorimeter would be able to record such an event if it does indeed occur.

MATERIALS AND METHODS

CALORIMETER

The adiabatic calorimeter used was constructed by Professor R. K. Burkhard (Department of Biochemistry, Kansas State University) for the study of solution thermodynamics. The instrument, similar to that outlined by Kitzinger and Benzinger (1960), consists of two thermopiles mounted on a rotating frame that enables the system to mix two or more substances rather easily (Figure 2). The calorimeter itself is located in a controlled environment where the temperature is maintained at 25°C. Special calorimeter cells were constructed by this University's Physics Shop. These cells contain a lengthwise partition that allow starch and water to be loaded and kept from contacting one another until the calorimeter was ready to record the water being added to the starch (Figure 3). It was discovered that a flat baseline could not be acheived unless the starch had been allowed to fully hydrate (a moisture content of approximately 20%) before loading in the calorimeter. Drier starch hydrates by absorbing water from the environment and producing heat, thus creating a sloped baseline. Hydrated native, ungelatinized starch gave no thermal response when water was mixed with the sample (Figure 4). It follows, then, that if there is a 'mixing' phenomenon, it should be seen when water is

added to the hydrated, heat treated starch, since this would be the only heat producing event taking place.

SAMPLE PREPARATION

Wheat starch was obtained from Midwest Solvents, Atchison, Kansas and was found to contain 10.4% moisture. Four moisture contents were studied - 10% (stock sample was used for this level), 30%, 50%, (a 1:1 water-to-starch ratio) and 66% moisture (a 2:1 water-to-starch ratio). The latter three starch samples were prepared by adding an appropriate amount of water to a pre-calculated amount of 10.4% moisture starch to give each sample 10 g of bone dry starch. The 66% moisture sample was placed in a glass sample jar while the other three were mixed in smaller glass vials and stirred with a spatula to help evenly distribute the moisture. After four days of refrigeration, the starches were loaded into aluminum moisture dishes (20mm x 48mm). The 10% and 30% moisture samples were loaded so as to fill the dish, leaving little air space above the material, and the other two moisture levels were filled half-way in the aluminum containers. A layer of teflon tape was placed over the bottom lip of the dishes to provide a tighter fit and to protect against moisture loss. All four samples were heated in an air oven maintained at 100°C for five hours. (The dishes were placed between two aluminum blocks as a safety measure.) After the treatment period, the samples were removed, cooled, and transferred to sample jars. The treated starch was frozen, freeze-dried ,and subsequently ground with a Wiley Mill using a 60 mesh screen. Fifty milliliter beakers containing a few grams of each ground sample were placed in a small dessicator over distilled water.

At least a one week equilibration period was given before the hydrated samples were run in the calorimeter.

CALORIMETRY

Sample weights ranged between 100 mg and 200 mg and were recorded to the nearest 5 milligrams. The treated starch was weighed in small plastic vials on a microbalance and then transferred to the calorimeter cells. Five milliliters of distilled water was then pipetted to the other side of the cell divider. Once loaded, the cell was allowed to sit overnight in the temperature controlled calorimeter room. The calorimeter itself was set at a sensitivity of 300 microvolts; one run was performed for each moisture level. Approximate sample weights were 170 mg for the 10% moisture heat treated starch, 125 mg for the 30%, 130 mg for the 50%, and 125 mg for the 66% sample.

RESULTS AND DISCUSSION

The 66% and 50% moisture samples formed gels upon heat treatment and both freeze-dried into very hard, resilient discs. It was not expected that these two starches would exhibit a mixing phenomenon in the adiabatic calorimeter since the gelatinization temperature range for both levels is below 100°C (57° - 67°C for 66% moisture and 57° - 90°C for the 50% moisture sample). If the granules are fully disrupted during gelatinization and sufficient water is present to completely dissociate those starch molecules possessing order, then no response should be seen when water is added to the sample in the calorimeter. The 30% sample was lumpy after the heat treatment and freeze-dried into small, hard, brittle clumps. Starch at 30%

moisture gelatinizes over a much wider temperature range, and at 100°C not all of the crystallites have been melted. If the mixing hypothesis is correct, a small thermal response should be seen when water is mixed with this starch in the calorimeter. This response, keeping in mind that the sample is fully hydrated, will consist of the excess water rapidly penetrating the granules - inducing swelling which will disrupt partially disordered crystallites. The added water will also dissociate starch chains that possess any residual order. These processes should result in an endothermic peak recorded by the calorimeter. The 10% sample retained its powdery nature through the heat treatment and freeze-drying so it was not ground. Starch at 10% moisture will not be completely gelatinized at this treatment temperature; in fact, melting of the crystallites has probably not yet begun at 100°C. Still, at the time, it was considered a possibility that the heat treatment would make these starch granules susceptible to these endothermic events upon the addition of excess water. However, no thermal event was recorded for either the 10% or the 30% moisture heat treated starch samples nor the 50% or 66% moisture levels (Figure 5).

In attempting to explain why no response was seen in the calorimeter, the following explanations readily come to mind. First is the consideration that a thermal event did occur, but the calorimeter did not record the response due to insufficient sensitivity of the instrument. This possibility can be discounted based on back calculations involving the actual data. The equation to calculate the energy associated with a peak recorded by this particular adiabatic calorimeter is as follows:

$$\text{Energy (mcal)} = \text{Peak Area (in 1/100th's of in}^2\text{) x Instrument Sensitivity (microvolts) x } 0.0010325 \text{ mcal/in}^2 \text{ microvolts}$$

For now, it will be assumed that the proposed mixing phenomenon could account for 0.5 mcal/mg of the loss in gelatinization enthalpy encountered with low moisture starch samples. Abbott et al. (1984) found that most of the starches they tested lost at least 0.5 mcal/mg in going from 66% moisture to just 33% moisture. Since the smallest sample size used was 125 mg, this figure will be used in the calculations. For a sensitivity of 300 microvolts, the calorimetric response would be:

$$125 \text{ mg} \times 0.5 \text{ mcal/mg} = \text{Peak Area} \times 300 \text{ microvolts} \times 0.0010325 \text{ mcal/in}^2 \text{ microvolts}$$

$$\text{Peak Area} \doteqdot 2 \text{ in}^2$$

Similarly, if smaller values are assumed for the energy of 'mixing' and the other variables are kept constant, the approximate responses would be:

If mixing were equivalent to:

0.3 mcal/mg, Peak Area $\doteqdot$ 1.2 in^2
0.1 mcal/mg, Peak Area $\doteqdot$ 0.40 in^2
0.05 mcal/mg, Peak Area $\doteqdot$ 0.20 in^2

Even if the mixing phenomenon would be expressed as an endothermic event with a magnitude of only 0.05 mcal/mg (approximately one-sixtieth that of the enthalpy of gelatinization), a peak would be recorded possessing an area of about 0.2 in^2. A response of this size could easily be seen, let alone the peaks associated with higher energies of 'mixing'. Thus, it would appear that instrument sensitivity was not a factor.

Another likelihood that cannot be discounted is that during the sample preparation process the mixing phenomenon was lost. For example, the thermal event could occur as the starch hydrates in the dessicator. Finally, it is very conceivable that no such mixing phenomenon exists, so no event could have occurred in the calorimeter. In light of the information now available, the last possibility seems most likely - that the mixing phenomenon, as described here, does not exist. Another experiment that appears to draw the same conclusion is that of Burt and Russell (1983), who were performing microscopic studies of starch at low moisture contents that had been heated in the DSC. To make sure that the observed loss of birefringence was due to the heat treatment and not to the placement of the starch in water to prepare the slide, some samples were added to ethanol or liquid paraffin instead of water. Although the granules were more difficult to observe, only a few granules were seen to possess weak birefringence, indicating that the loss of order occurred during heating in the DSC and not upon subsequent addition of the starch to water.

Other findings may indicate that the theoretical basis for the mixing phenomenon, that ΔH decreases as the amount of water in the sample decreases, may not be correct. Abbott et al. (1984) found that gelatinization enthalpy does not uniformly decrease for all starches from water-to-starch ratios of 2:1 to 1:1 to 0.5:1. Most cereal starches exhibited a decline in ΔH as less water was added to the sample, but potato and buffalo gourd starch only experienced a slight drop in enthalpy over the moisture range studied. Most of Donovan's (1979) work centered on potato starch, although he concentrated on samples with a

lower moisture content than the 0.5:1 water to starch ratio (33% moisture) used above.

Many good explanations exist to account for the observed loss in gelatinization enthalpy. Wendlandt (1974) points out that, due to physical limitations, a differential scanning calorimeter will sample only a portion of the energy taken in or given off as a phase transition occurs. Thus, an event which occurs over a narrow time frame, such as the low temperature gelatinization peak, will have a larger percentage of the total energy recorded than if the transition takes place over a broad temperature range, as is the case with the high temperature melting endotherm. The difference in temperature ranges for the excess water gelatinization peak ($10^{o}C$-$15^{o}C$) and the low moisture melting endotherm (possibly up to $40^{o}C$-$50^{o}C$ in duration) is quite large, hence machine bias could account for a significant portion of the reduction in enthalpy.

Another major problem associated with determining enthalpy values concerns the construction of a suitable baseline for low moisture starch thermograms. For these samples, the baseline begins to drift appreciably during the latter half of the thermogram (see Figure 1). Hence, even though the melting endotherm appears to decrease in size with lower moisture contents, defining peak area becomes less certain, especially due to the gentle slope of the leading edge. After the recorder trace gradually outlines the broad melting endotherm, the peak may run into the amylose-lipid complex peak or the upper temperature limit for the sample run may have been reached. These factors make the construction of a baseline for this higher

portion of the curve extremely difficult. Thus, peak measurements for low moisture samples would have a tendency to be less accurate (Wendlandt 1974); this could result in smaller values for enthalpy.

CONCLUSION

From the results obtained in this experiment, and, based on additional information, it can be concluded that the 'mixing phenomenon' as described above does not exist. The enthalpy of gelatinization should be thought of as the result of multiple thermal events occurring within the same time frame. Crystallite melting, granule swelling, formation of the amylose-lipid complex, and hydration of the starch chains are some of these events, but their individual contributions to the energy associated with the gelatinization phase transition have not yet been identified. Hydration is discussed further in the chapter on differential scanning calorimetry; it is thought to exert a definite influence on how the energy associated with gelatinization is expressed. Any energy from swelling and disruption of the granule may add to the endothermic energy associated with melting of the crystallites. It may not be possible to separately study these fractions since all three processes occur within the same time frame and are no doubt dependent on each other. Based on the above discussion, it probably can also be concluded that these phenomena are not expressed unless heat is being applied to the system. In other words, if water is added to a low moisture starch sample that has been only partially gelatinized, any 'dry' starch molecules will

hydrate, but no calorimetrically detectable granule swelling or denaturation will take place. Finally, further investigation of low moisture starch systems is needed to ensure that the observed decrease in enthalpy associated with a decrease in water content is not an artifact, and if it is indeed a real phenomenon, why does it occur.

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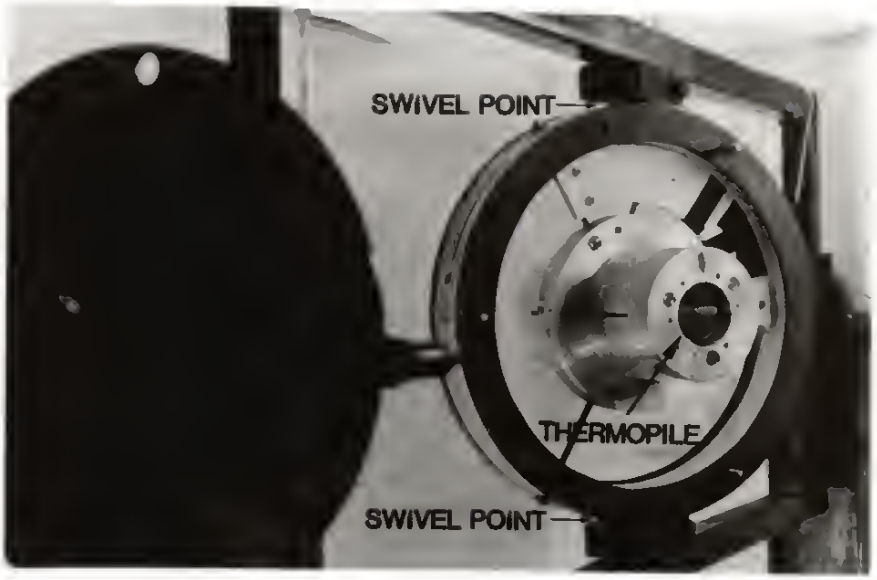
Figure 2. Photographs of the adiabatic calorimeter that was used to test heat treated starch for residual order. The frame can be rotated with the thermopiles either parallel or perpendicular (see swivel points) to the frame.


SWIVEL POINT
THERMOPILE
SWIVEL POINT

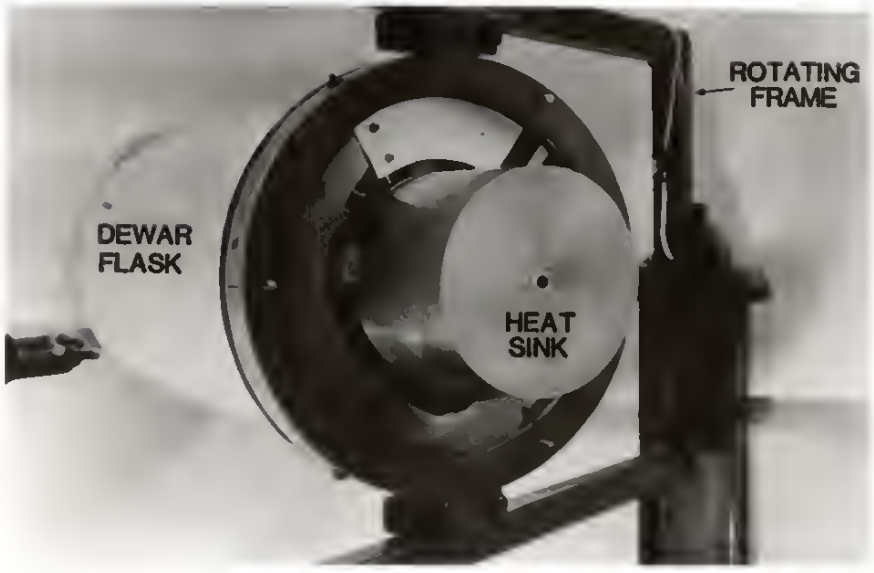

ROTATING FRAME
DEWAR FLASK
HEAT SINK

Figure 3. Photograph of the calorimeter cells in which the starch samples were mixed with water.

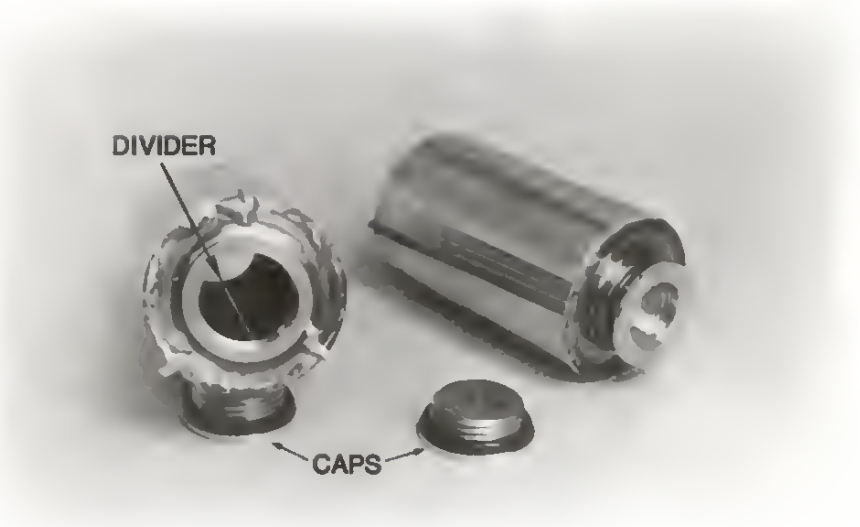

DIVIDER
CAPS

Figure 4. Adiabatic thermograms for starch showing: a) the effect on the baseline when starch absorbs water, b) no response when water is mixed with fully hydrated starch, and c) a typical hydration exotherm for approximately 50 mg of 10% moisture starch mixed with water. (The starch used in c) was isolated from the moisture in the calorimeter cell, unlike a) and b). Sensitivity of the calorimeter was 1000 microvolts in c) compared to 300 microvolts for a) and b). The spikes in the baseline labeled MIX are the responses generated by rotating the calorimeter to mix the water and the starch. These mixing motions are usually performed twice to ensure that the reaction is complete.)

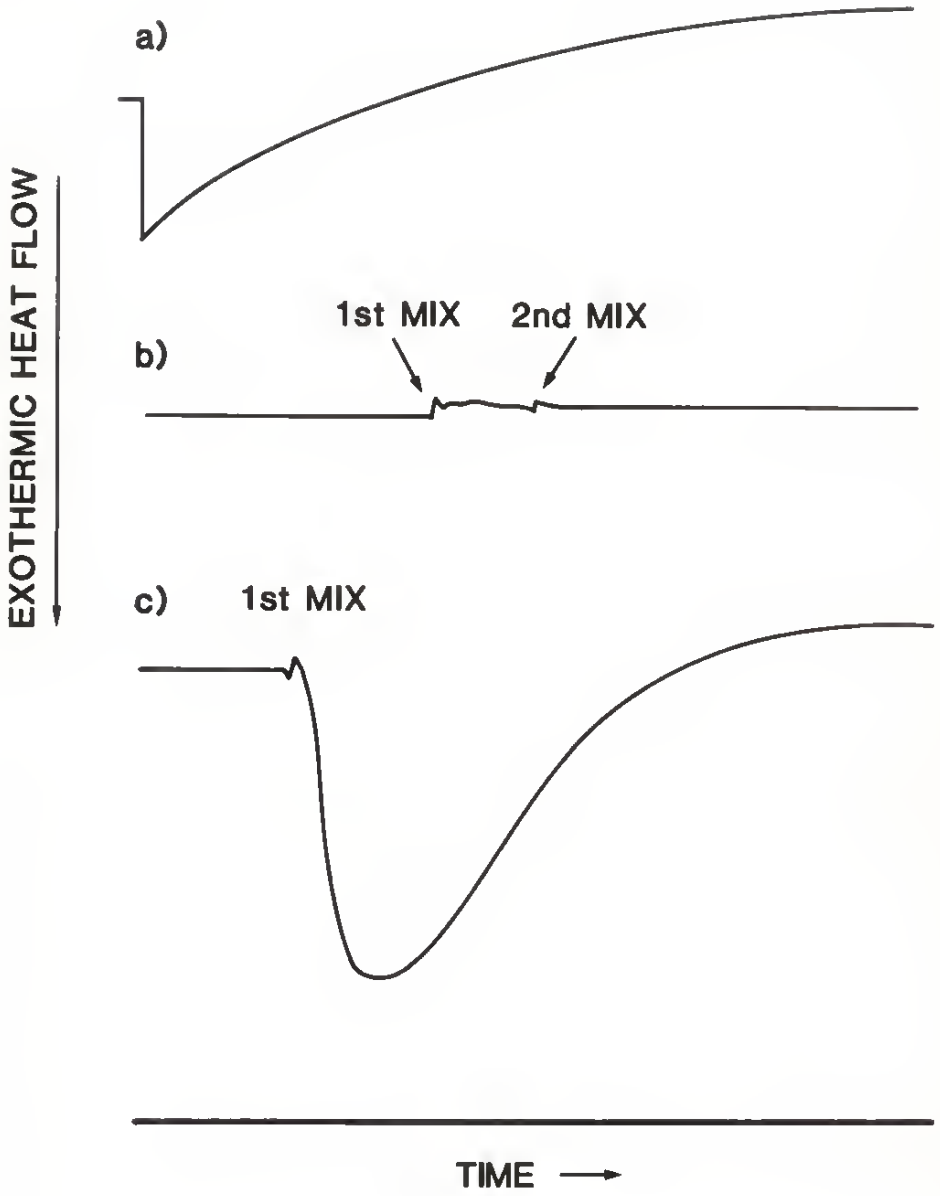
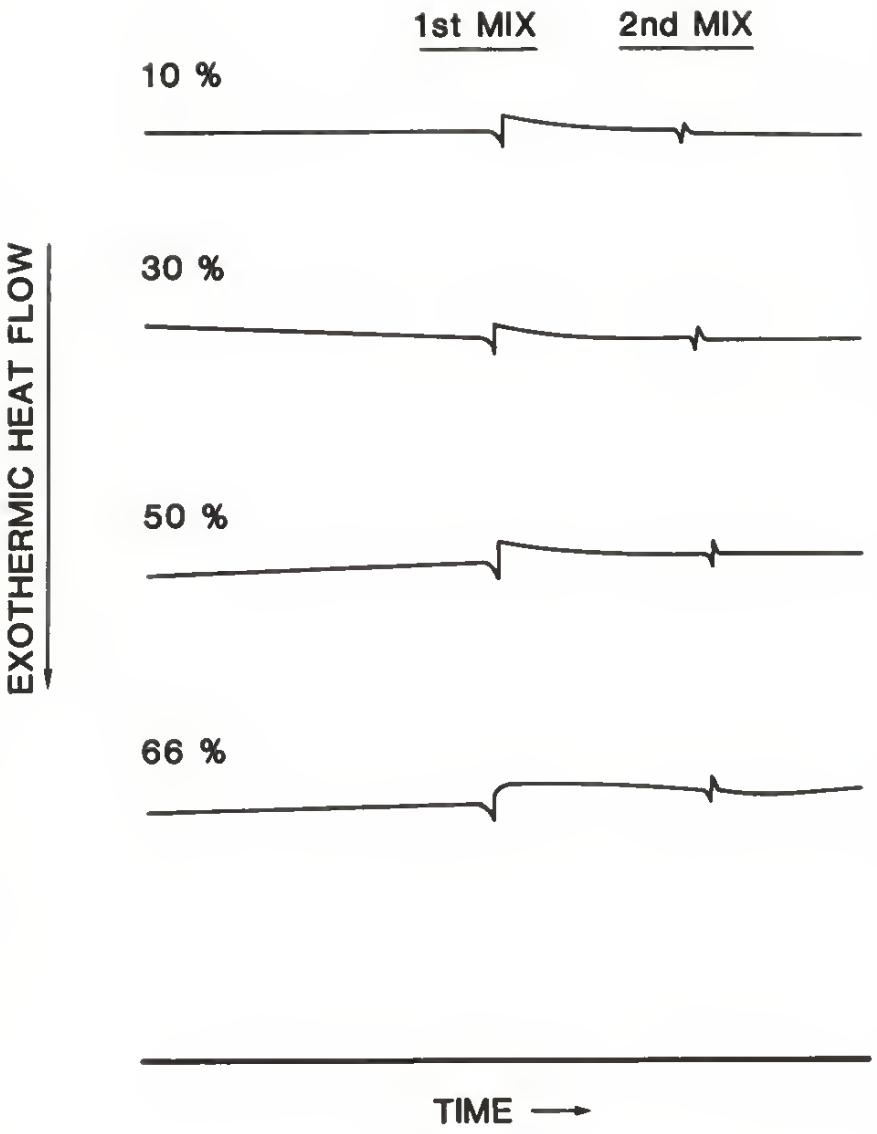

a)
b)
1st MIX
2nd MIX
c)
1st MIX
EXOTHERMIC HEAT FLOW
TIME

Figure 5. Adiabatic thermograms showing the addition of excess water to hydrated starch samples (1st MIX) that were heat treated at moisture levels of 10%, 30%, 50%, and 66%.


1st MIX
2nd MIX
10 %
30 %
50 %
66 %
EXOTHERMIC HEAT FLOW
TIME

CHAPTER 3

DIFFERENTIAL SCANNING CALORIMETRY

INTRODUCTION

The differential scanning calorimeter (DSC) has been used extensively in the last fifteen years to study the interaction of starch with water at elevated temperatures. Most of this work has concentrated on the various aspects of starch gelatinization; the instrument has proven to be a very useful tool for the study of this order-disorder transition. The DSC has once again been employed to study the starch granule, as it has recently been suggested that starch exhibits another transition common to other semi-crystalline polymers.

Maurice et al. (1983) have pointed out that the amorphous areas of starch granules should undergo a glass transition upon heating. They contend that this second-order transition is a part of, or occurs just before gelatinization and that the temperature at which the glass transition will occur is dependent on the water content of the starch. DSC analysis of starch does not indicate where the glass transition occurs - not only is the event masked by the large gelatinization endotherm, but the heating rates used for normal starch analysis are probably too fast to pinpoint a temperature for the transition. Starch thermograms do, however, exhibit a baseline shift after the gelatinization peak which is indicative of the change in heat capacity that accompanies a glass transition (Billingham and Jenkins 1972). According to polymer literature, most, if not

all, semi-crystalline polymers undergo a glass transition. However, in most instances, a wide difference exists between the glass transition temperature and the melting temperature for any given polymer. As a general rule, the glass transition temperature for many polymers is approximately two-thirds of the melting temperature (expressed in absolute temperatures) (Van Krevelen 1976). Thus, it is conceivable that a glass transition can occur in starch at a temperature lower than its excess water crystallite melting temperature and that altered granular integrity - or other factors - due to gelatinization is responsible for the change in specific heat of the starch and thus, the observed baseline shift. For convenience, the glass transition temperature and the onset temperature of gelatinization will be referred to as Tg and Tm, respectively.

The phenomenon of annealing may provide a method to determine if, and at what temperature, a glass transition does occur in starch granules. Annealing of crystallites does occur; this was first reported by Gough and Pybus (1971) who noted that wheat starch, treated with water at 50°C for three days, gelatinized at a higher and more sharply defined temperature than an untreated starch sample. For polymers, Williams (1975) states that, "... both the number and size (of spherulites) can be increased by annealing at a temperature between the melting point and the glass transition temperature, ...". If no annealing can occur below the glass transition temperature, then Tg can be approximated by the temperature at which the phenomenon begins. One possible problem, however, is that starch behaves differently under annealing conditions than do most polymers. Starch

crystallites are rather short, but lengthening of these crystals is not likely. This would no doubt be due, in part, to the large amount of reorientation required to align the amylopectin chains so that lengthening could occur. Moreover, the average chain length for amylopectin molecules is only 20 - 30 glucose residues (Shannon and Garwood 1984); this in itself almost precludes any lengthening of the crystallites from occurring. Thickening of the ordered regions is possible and energetically more favorable, but apparently does not occur either. What seems to take place is that the crystals become more perfect; this is expressed not as an increase in melting enthalpy, but as an increase in Tm and a narrowing of the temperature range for gelatinization (Hoseney 1984). Although these are significant differences (crystallite enlargement versus crystallite perfection), the use of annealing to study the glass transition in starch should still be appropriate.

MATERIALS AND METHODS

Starch samples were heated in a Perkin-Elmer DSC-2 (with an Intracooler II) and the thermograms recorded on a Perkin-Elmer Model 56 chart recorder. Wheat starch, obtained from Midwest Solvents, Atchison, Kansas, was stored in a glass sample jar and used exclusively for all runs. The starch was found to have a moisture content of 10.4% (w.b.). All samples tested in the calorimeter, unless otherwise indicated, were at a 1:1 water-to-starch ratio (water content of 50%). To prepare a sample, approximately 3 milligrams of starch, within 0.1 mg, was weighed into a Perkin-Elmer DSC aluminum sample pan on a microbalance.

To bring the wheat starch up to the desired moisture content, the following procedure was used. All starch weighed into the pans was assumed to be 10% moisture. Thus, for a 3.000 mg sample, 2.700 mg would be dry starch. Therefore, to achieve a 1:1 ratio, the total starch/water sample would weigh 2.700 mg x 2 = 5.400 mg, or, 2.4 mg of water must be added. Water was added to the pan through a microliter syringe, the pan placed back on the microbalance, and any excess water allowed to evaporate. When the weight approached the desired value, the pan was removed from the balance and hermetically sealed. The pan and sample were weighed once more to record the final weight, which allowed calculation of the actual amount of water added.

All samples were tested with a heating rate of 10°K per minute between 280°K and 400°K. Another aluminum pan, containing an appropriate amount of aluminum to balance the heat capacity of the sample, was used as a reference. All peaks were measured by planimetry, and the areas converted to enthalpy values.

RESULTS AND DISCUSSION

The first set of starch samples was run at a water-to-starch ratio of 1:1 and a one hour waiting period was observed after sealing the aluminum pan before each sample was run in the DSC. These samples were heated to various temperatures between 300°K (27°C) and 329°K (56°C), held at these temperatures for thirty minutes, then cooled and rerun as in a normal scan, from 280°K to 400°K (7°C - 127°C). The gelatinization results from the scans of the heat treated starch are listed in Table 1 and represented graphically in Figure 6. Even though only a few measurements

have been taken at each temperature, certain characteristics are evident from the data. At each holding temperature, the enthalpy of gelatinization values are not very reproducible. It would also seem obvious that once a sample has been heated to 327°K (54°C), melting of the crystallites has definitely begun because ΔH declines rapidly past 325°K (52°C). A precise estimate of where melting begins is not available from this set of data. In looking at the graph of enthalpy values, a slight tendency may exist for gelatinization enthalpy to increase due to the thirty minute holding period as compared to samples that were allowed to equilibrate one hour after sealing but were not heat treated in the manner described above. If this increase is indeed real, it would seem to occur even at 300°K (27°C), which is only a few degrees above room temperature. This observation may not have a sound basis, since only a few measurements were taken at each treatment temperature and the distribution of values cannot be discounted as just a random scattering of data when compared to the average enthalpy for starch that was not heat treated. For these nontreated samples, a large amount of variability is associated with these values also (Table 2). Although the average of 2.89 cal/(g of dry starch) is below most of the gelatinization enthalpies for the treatment runs, the standard deviation for these eight measurements (±0.09 cal/g) would indicate that these values for the heat treated starch are not radically different than those for untreated starch. Before these results can be analyzed for crystallite annealing and the presence or absence of a glass transition, a more thorough understanding of the distribution of gelatinization enthalpies is

necessary.

To check further into the wide range of enthalpy values seen when 50% moisture starch is heated in the DSC, ten samples were prepared and run as soon as possible after sealing the aluminum pan. (This turned out to be approximately eight minutes from the time the pan was sealed to when the heating program began in the DSC.) The average ΔH for this group of samples was 2.84 ± 0.10 cal/(g of dry starch), which is slightly lower than the samples which were held for one hour before running, but with about the same amount of variability (Table 2). Nine more samples at the same moisture content were run, except these starch-water mixtures were allowed to sit for 24 hours before they were heated in the calorimeter. These samples possessed a somewhat higher average, 3.00 ± 0.04 cal/(g of dry starch), but, more importantly, are considerably lower in variability. At this time, the most pertinent question is whether these three different time periods used between sealing the aluminum pans and running the samples in the DSC will actually produce different values for the enthalpy of gelatinization at this moisture content. To analyze this, a very simple statistical tool, the Empirical Rule, will be used. This rule states that all, or almost all, measurements from a normally distributed population will fall within three standard deviations of the mean (Mendenhall 1979).

The assumption that must be made, of course, is that the distribution of gelatinization enthalpies for this particular starch, at this water content, under similar conditions, will be bell shaped, that is, centered about the average value for each

particular waiting period (0, 1, or 24 hours). Constructing frequency histograms for each of the three treatments does not provide any real indication as to whether these populations are normally distributed, but this would probably be due to the low number of observations taken for each waiting period. A better way to characterize the data may be to use the Empirical Rule once again. In addition to stating that all or nearly all measurements will be found within three standard deviations of the mean, it also says that approximately 68% of the measurements will lie within one standard deviation of the mean and that approximately 95% will lie within two standard deviations. For all 27 observations taken for the three treatments, every ΔH value lies within two standard deviations of its respective mean. Within one standard deviation, 20 out of the 27 measurements lie inside this range for a 74% total (Table 2). Considering the estimates provided with the Empirical Rule, the gelatinization enthalpy values for all the treatments seem to follow these guidelines for a normal distribution. Although many, many observations must be taken to prove this, it would appear to be a fairly safe assumption to say that the ΔH's are normally distributed in all cases.

Now to reconsider the earlier question - whether waiting 24 hours before running a starch sample at 50% moisture will produce a different value for ΔH compared to running the sample soon after sealing the pan. Referring to Table 2, values within three standard deviations for the samples run immediately after sealing would include any in the range 2.54 - 3.14 cal/g. For the starch samples that sat 24 hours before being run, this range is 2.88 -

3.12 cal/g. In comparing these two treatments, it can be said that the 24 hour treatment contrasted with the no hold time treatment is probably not different because all ΔH values for the 24 hour group fall within the probable range of values for the no time group. In other words, it cannot be discounted that the enthalpies for the 24 hour treatment are simply within the normal scattering of data for the no hold treatment, i.e., holding starch for these two different time periods would not result in significantly different enthalpy values for gelatinization. However, if we look at the no hold treatment versus the 24 hour treatment, a different result is obtained. The average ΔH of the no time group is not even within the three standard deviation range for the one day hold group (2.84 cal/g average, 2.88 - 3.12 cal/g range). The scattering of data points is much narrower for starch samples allowed to equilibrate for this extended period, and it is highly unlikely that the normal scattering of data would include many of the values associated with the no time treatment. Thus, from this analysis, it can be concluded that allowing starch to sit in the aluminum pan for 24 hours will result in a larger value for gelatinization enthalpy than by just running the sample after it is sealed. A similar conclusion is reached when comparing the samples held for one hour before running in the DSC to the 24 hour group; some values obtained with the one hour treatment are outside the range of expected values for the one day holding treatment. Hence, a good possibility exists that these two treatments will give a different endothermic response.

Further support for the notion that gelatinization enthalpy

increases with the length of time between sealing the aluminum pan and heating in the DSC can be found with two additional sets of thermograms. In these two series, the time that the starch/water samples were kept in the pan before heating was increased. The gelatinization results are presented in Table 3. In both instances, ΔH appears to increase with the amount of time the mixture is allowed to equilibrate. In looking at the two sets of data, it becomes clear that cross - comparison of these series of runs, which were performed on two different days, can give puzzling results. The reason so much difference could exist is not clear (2.85 cal/g for one six hour waiting period versus 3.12 cal/g for the other), but it may involve day-to-day variations within the instrument or the starch.

In trying to explain the increase in ΔH with equilibration time in the sample pan, it is important to realize that a time, and quite possibly a temperature, dependent event is taking place in the starch at room temperature and at elevated temperatures. Two possible explanations exist as to why the enthaply of gelatinization increases as a starch sample at 50% moisture is held for a period of time at elevated temperatures. One possibility is that the starch crystallites are annealing when exposed to these temperatures. If the crystalline regions are increasing in size, this could explain an increase in the energy required to disorder the system. An alternative hypothesis, one which can explain both the room temperature and the higher temperature results, involves hydration of the starch granules. When water is added to starch, even in excess amounts, time is required for the water molecules to penetrate the granule's

amorphous areas and hydrate accessible starch chains. Obviously, the longer the system is allowed to equilibrate, the more complete will be the hydration. It also seems likely that hydration will proceed at a more rapid rate when the equilibration period occurs at an elevated temperature. How then, does granular hydration result in a larger enthalpy value?

Both Donovan (1979) and Evans and Haisman (1982) have suggested that the enthalpy associated with gelatinization is the result of multiple thermal processes occurring within the same time frame. In essence, the gelatinization endotherm represents the difference between the endothermic energy associated with the transition (melting of the crystallites, granule swelling and denaturation) and the exothermic energy associated with the transition (hydration of starch molecules, formation of the amylose-lipid complex (Kugimiya et al.1980)). This being the case, a starch/water sample held for a period of time before it is run in the DSC would exhibit a larger endothermic response than a starch sample treated soon after the addition of water since a more complete hydration of the granule's amorphous areas has taken place in the former sample. The exothermic portion of the gelatinization process (hydration) will be smaller, allowing more of the endothermic events to be recorded. This same masking effect has recently been reported, from a study on the heat of immersion of starch, to occur between hydration of starch molecules (exothermic event) and granular swelling (endothermic event) (Wurster et al. 1984).

Some starch samples heated soon after sealing the aluminum pan possess the larger ΔH associated with long equilibration

periods. This does not discount hydration as a realistic explanation of why ΔH increases; for whatever reason, some samples can apparently experience a more rapid hydration compared to other samples run under nearly identical conditions. Here, it is important to note that although samples run immediately may have larger ΔH values, samples that have equilibrated for any length of time show no tendency to exhibit enthalpy values down in the range of the no time holding samples. Thus, the factor(s) that control hydration can greatly influence the starch/water mixture soon after the addition of water, but the effect apparently levels out and each sample will be affected to the same extent given sufficient time. If annealing is responsible for this increase, then the data indicates that annealing of starch can proceed at room temperature upon the addition of water - suggesting that Tg is below room temperature. While this is not out of the question, the hydration phenomenon appears to be a more complete explanation as to why the increase in enthalpy occurs.

A series of samples tested at a 2:1 water-to-starch ratio also supports the hydration explanation. These samples, held various times before running in the DSC, showed no tendency for ΔH to increase with longer equilibration periods as with the 1:1 samples (Table 3). This would indicate that the increase in ΔH seen for the 50% moisture samples would not be due to annealing because annealing would be just as likely to occur in starch with an excess of water. Apparently, if an excess of water is present, hydration will be completed sooner than with samples in a limited water situation, which would be expected.

It is now possible now to better analyze the results from the samples that were held for thirty minutes at various temperatures below gelatinization, then rerun. Examining peak onset temperature and peak shape may provide important clues to the annealing - hydration question. An annealed sample would, most likely, contain starch granules whose crystallites are more perfect. Marchant and Blanshard (1980), as well as Gough and Pybus (1971), found that this change corresponds to a higher initiation temperature and a more sharply defined temperature range for gelatinization. With the DSC, this would be seen as a narrowing of the endothermic peak along with an elevated onset temperature associated with gelatinization. If hydration is the only process responsible for the increase in enthalpy over time, the shape of the gelatinization endotherm should not be significantly altered, just slightly larger, and the peak should have a normal onset temperature. For the scans of starch samples held at 300°K, 305°K, and 310°K, the inherent shape or onset temperature of the gelatinization endotherm does not appear to change. However, for the 320°K (47°C) and 325°K (52°C) samples, the peak onset temperature of the second run is shifted upwards approximately 2°K and 3.5°K, respectively, and the gelatinization peak is slightly narrowed (Table 1 and Figure 7). It might be argued that these changes begin at 315°K (42°C); although the curves for this temperature are not noticeably different, it appears that the onset temperature shift is beginning to occur.

Even though a small number of observations were taken, some tendencies due to the treatments can be seen and should be presented for discussion. In Figure 6, it would appear that the

range of ΔH values for each holding temperature distinctly broadens for 315°K and 320°K. The average enthalpy seems to increase through 315°K and attains the highest average value at 320°K before melting begins. The decrease in ΔH at 325°K is interpreted as the first indication of crystallite melting; the large drop in enthalpy for 327°K (54°C) and 329°K (56°C) supports this assumption. If annealing is indeed occurring at 315°K or 320°K, it is taking place in addition to hydration. The additive effects of these two processes (slight rearrangement of the crystallites may facilitate hydration) could account for the larger increase in ΔH near the melting temperature of the crystallites. An increased complexity of the system could account for the greater variability associated with the 315°K and 320°K treatments. If melting of the crystallites has started at 325°K, then some annealing must take place here since this temperature is no doubt within the Tg to Tm range of some of the crystallites. In addition, the possibility exists for partially disrupted crystallites to recrystallize at this annealing temperature. For the 300°K, 305°K, and 310°K samples, the increased ΔH values - comparable in magnitude to letting the starch/water mixture equilibrate for a long period of time - are attributed to hydration. For 305°K and 310°K, it could be assumed that the slight increase in temperature is responsible for the relatively large ΔH, but this explanation will not hold true for the 300°K (27°C) observations. 300°K is only a few degrees (1°- 3°C) above room temperature where it took considerably longer than 1-1/2 hours to acheive similar values. It can only be suggested that the factor(s) affecting hydration

are favorably influenced by the conditions in the DSC during the thirty minute hold.

It is likely that these changes in the gelatinization endotherm at 320°K and 325°K are due to annealing of the crystallites. However, the excess water gelatinization mechanism is not understood and it is entirely possible that the endothermic response can be altered by these heat-time treatments without the starch crystallites having been annealed. Evidence does exist, though, that annealing occurs in starch granules (Gough and Pybus 1971, Marchant and Blanshard 1980). Data from 50% moisture samples heated to and held at 345°K (72°C) (within what is generally considered the melting portion of the curve) for no time, twenty, forty, and sixty minutes then rerun, support the idea that starch can be annealed (Table 4 and Figure 8). After an initial decrease in enthalpy of the treated starch from no hold time to twenty minutes, ΔH of melting the remaining crystallites increases linearly from twenty to forty to sixty minutes held at 345°K. Compared to the broad peak observed when the sample was stopped and immediately rerun, heat treatment produced a narrower peak with an onset temperature shifted 7°-8°K higher (Figure 9). In this instance, annealing of crystallites would seem to be the most probable cause for these changes. It has been suggested, however, that gelatinization enthalpy should not increase for starch that has been annealed (Hoseney 1984). The prevailing thought is that the crystallites do not enlarge, but reorganize to form more perfect crystals when starch granules are exposed to annealing conditions. The conditions at 345°K are different than those found with annealing ungelatinized starch,

i.e., more starch chains are available and have more mobility to participate in annealing. Peak area definitely increases with the length of heat treatment at 345°K, indicating, most likely, that the crystallites are enlarging. A way to explain this effect without invalidating the hypothesis for crystallite annealing is to postulate that crystallites which are not completely disordered when the heating program is stopped can possibly recrystallize to some extent. The chains involved in these particular crystals would still be somewhat oriented and it would seem likely that, given time, enough thermal energy is present in the system to allow some of these chains to reassociate. This would result in a larger ΔH value with increased holding periods at 345°K. At the same time, the remaining crystals are annealing - becoming more perfect - as evidenced by the narrowing of the peak and its shift to higher temperatures.

The decrease in enthalpy from zero to twenty minutes of holding time may indicate that some factor in addition to just a melting phenomenon is in evidence since ΔH should not drop if annealing is the only phenomenon occurring. It was thought that the 'mixing' phenomenon - discussed in the chapter on adiabatic calorimetry of wheat starch - could account for this loss in ΔH. Further work has since discounted the existence of the 'mixing' effect. For the run with no holding time at 345°K, a period of two to three minutes elapsed before the second heating scan was begun at 280°K. In addition, six and one-half minutes were required to reattain 345°K. This amount of time makes it unlikely that hydration or a similar process is responsible for

the initial decrease from zero to twenty minutes. Interestingly, the peaks associated with the first thermogram to 345°K and the second thermogram to 400°K for the no hold sample, when added together, are roughly equivalent to a 1:1 starch sample scanned in a normal fashion. In other words, no enthalpy is lost for this treatment; not until the sample is held at 345°K will the ΔH associated with events beyond 345°K decrease.

In excess water, gelatinization is thought to be temperature dependent and not time dependent. For example, a starch sample stopped in mid-gelatinization will not continue to gelatinize even if held at that temperature for a long period of time. If this sample were cooled and reheated, gelatinization will resume at the temperature where the transition was stopped, not at the original onset temperature for gelatinization. Marchant and Blanshard (1978) did report a lag in gelatinization response when they heated starch in small temperature jumps of 2°-3°C. However, they found no such lag when larger jumps of 8°-10°C were used. The data from the present study may indicate that melting of starch crystallites beyond the excess water gelatinization peak may indeed be time dependent. The effect, that holding 1:1 starch at 345°K may melt some crystallites, appears to occur in entirety within the first twenty minutes (probably within the first few minutes) at which time crystallite annealing will begin to predominate. Finally, the holding temperature of the DSC does not appear to vary significantly, so the loss in ΔH could not be explained by a random fluctuation in the temperature of the instrument causing some of the crystallites to melt.

CONCLUSION

When a half water - half starch system is held for thirty minutes at elevated temperatures, the resulting energy required for gelatinization increases. An increase in enthalpy was also shown to occur as a function of the amount of time a starch sample is allowed to sit in the aluminum pan before being run in the DSC. The latter instance is explained as a hydration phenomenon. For starch samples held at 300°K, 305°K, and 310°K, hydration also appears to be the best explanation for the noted increase in ΔH. But, at 320°K, and possibly at 315°K, another phenomenon begins that shifts the onset temperature of gelatinization higher and changes the shape of the gelatinization peak. These effects are attributed to annealing of the crystallites within the starch granules. The question thus becomes, does the annealing effect precede the order - disorder transition? If so, this would indicate that the glass transition temperature is below the gelatinization temperature. Peak onset temperature for the samples that were not heat treated, 333.5°K or about 60°C, is at the upper limit of the range of temperatures normally found in the literature for wheat starch gelatinization. This is primarily due to the thermal lag experienced when using a fairly rapid heating rate in the DSC. The temperature that will be recognized as the lowest temperature at which gelatinization can begin will be that found by Bowler et al. (1980). They studied the morphological changes of starch granules during heating in water and found that irreversible swelling of the granule associated with gelatinization begins at approximately 50°C. Thus, any annealing effects that can be seen below 50°C

(323°K) would indicate that the glass transition temperature is below the gelatinization temperature. According to the data, annealing has definitely begun at 320°K (47°C) and, most likely, begins between 315°K (42°C) and 320°K (47°C). This would be 3°- 8°C below the temperature for the initiation of gelatinization. A difference of this magnitude effectively eliminates the possibility that an error in the temperature calibration of the DSC could be responsible for erroneously low values associated with the annealing effect.

The evidence here does not conclusively prove that starch undergoes a glass transition, but changes within the granules that can be attributed to the presence of such a phenomenon definitely occur, and are seen to take place well before the onset of gelatinization.

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Table 1. Starch Gelatinization Data After a Thirty Minute Annealing Period.

Annealing Temperature (^{o}K)	Observation (1) $\Delta H^{1}/ T_{o}^{2}$	(2) $\Delta H / T_{o}$	(3) $\Delta H / T_{o}$	Average
300	3.03/333.5	3.04/333	3.16/334	3.08/333.5
305	3.05/333.5	2.98/333.5	2.92/333.5	2.98/333.5
310	2.99/333.5	3.07/333.5	2.95/334	3.00/333.5
315	3.09/333.5	2.85/333.5	2.95/334	
	3.28/334.5[3]			3.04/334
320	2.98/335.5	3.33/334.5	3.05/336	3.12/335.5
325	2.84/337.5	3.03/336.5	2.76/337.5	2.88/337
327	2.59/338.5			
329	2.32/340			

[1] ΔH: Gelatinization Enthalpy (cal/g)

[2] T_{o}: Onset Temperature of Gelatinization (^{o}K)

[3] Four Observations Were Taken at 315^{o}K

Figure 6. Distribution of gelatinization enthalpy values for wheat starch annealed for thirty minutes at various temperatures. All samples were at a 1:1 water-to-starch ratio. The average enthalpy values for each annealing temperature are represented by the dotted line.

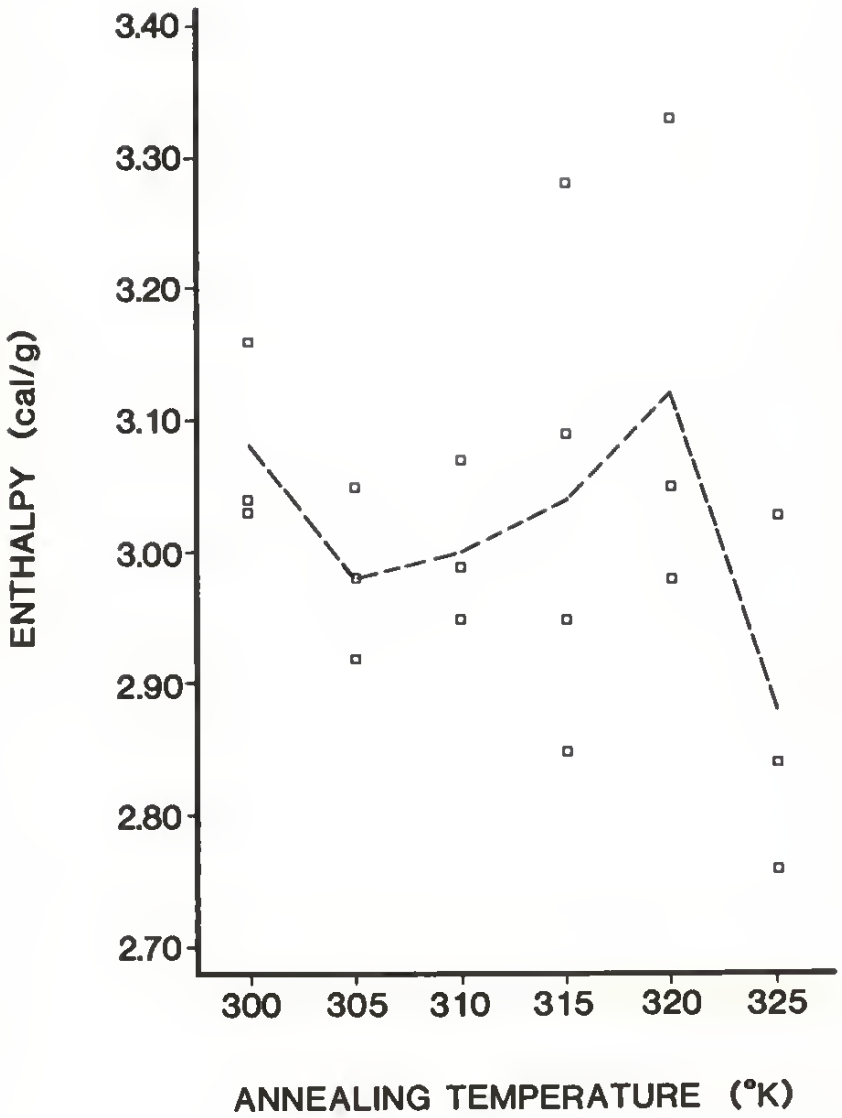

3.40
3.30
3.20
3.10
3.00
2.90
2.80
2.70
ENTHALPY (cal/g)
300
305
310
315
320
325
ANNEALING TEMPERATURE (°K)

Figure 7. DSC thermograms illustrating the effect of annealing on the gelatinization endotherm of starch. An unannealed sample is shown in a), whereas b) and c) were annealed at 320^{o}K and 325^{o}K, respectively, for thirty minutes.

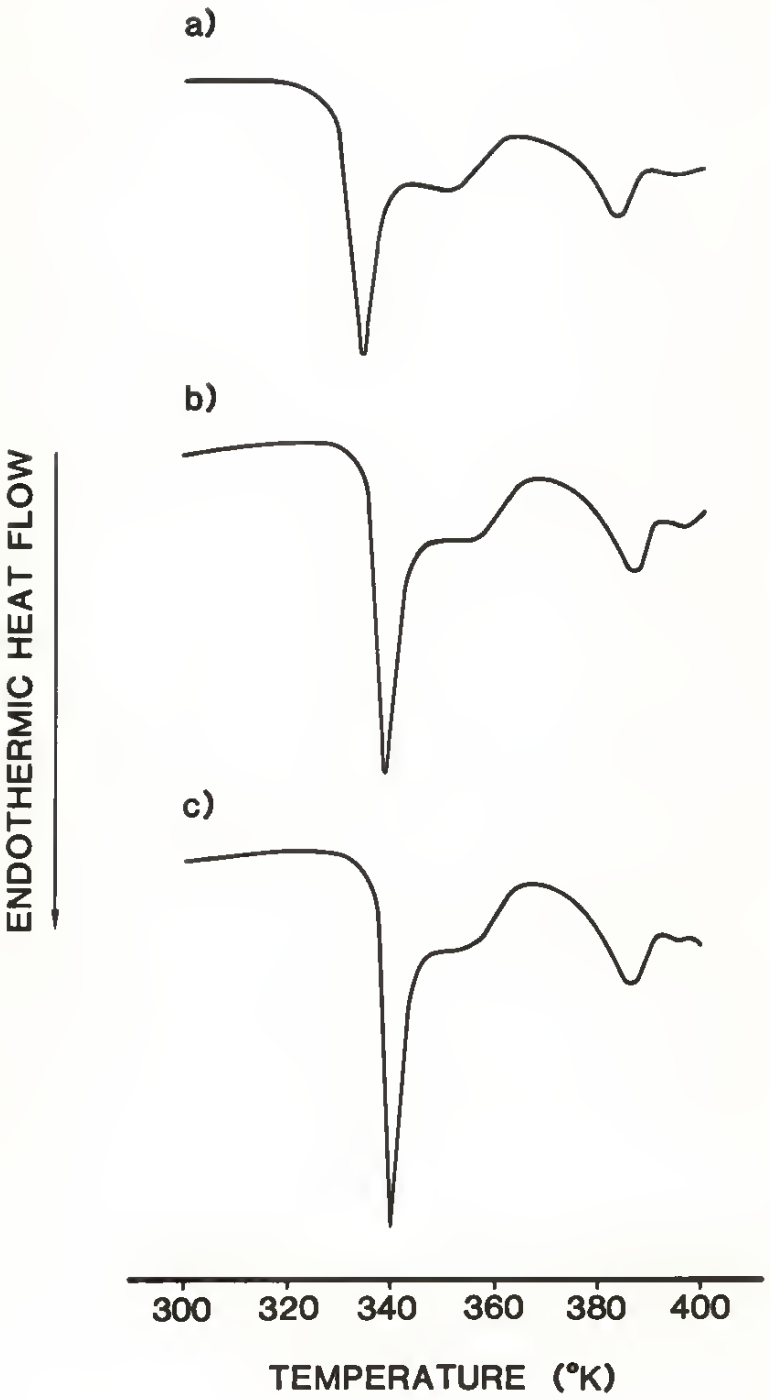

a)
b)
c)
ENDOTHERMIC HEAT FLOW
300
320
340
360
380
400
TEMPERATURE (°K)

Table 2. Enthalpy Values for 50% Moisture Starch Held in the Aluminum Sample Pan for Varying Amounts of Time Before Being Run in the DSC.

Observation	ΔH (cal/g) no waiting period	ΔH (cal/g) 1 hour waiting period	ΔH (cal/g) 24 hour waiting period
1	2.86	2.83	3.04
2	2.97	2.75	3.05
3	2.88	2.95	2.95
4	2.97	2.92	3.03
5	2.74	2.80	3.03
6	2.77	2.91	2.96
7	2.93	2.95	3.02
8	2.76	3.03	2.97
9	2.72		2.97
10	2.76		
Average	2.84	2.89	3.00
Standard Deviation	0.10	0.09	0.04

Table 3. Enthalpy Values For 50% and 66% Moisture Starch Held in the Aluminum Sample Pan for Increasing Amounts of Time Before Being Run in the DSC.

	Hours held before running	ΔH (cal/g) (1:1)	ΔH (cal/g) (2:1)
	2	2.92	
TRIAL 1	4	3.02	
	6	3.12	
	0		3.04
	2	2.83	3.01
	4	2.93	2.95
TRIAL 2	6	2.85	2.93
	8	3.00	2.97
	10	2.96	2.93
	12	3.06	3.00

Table 4. Enthalpies Associated With Melting the Annealed Crystallites After Holding the Starch at 345°K.

Minutes held at 345°K	ΔH (cal/g) of the residual crystallite peak
0	0.74
20	0.44
40	0.50
60	0.56

Figure 8. Graph showing the increase, over time, in energy required to melt starch crystallites that had been annealed at 345°K at a water-to-starch ratio of 1:1.

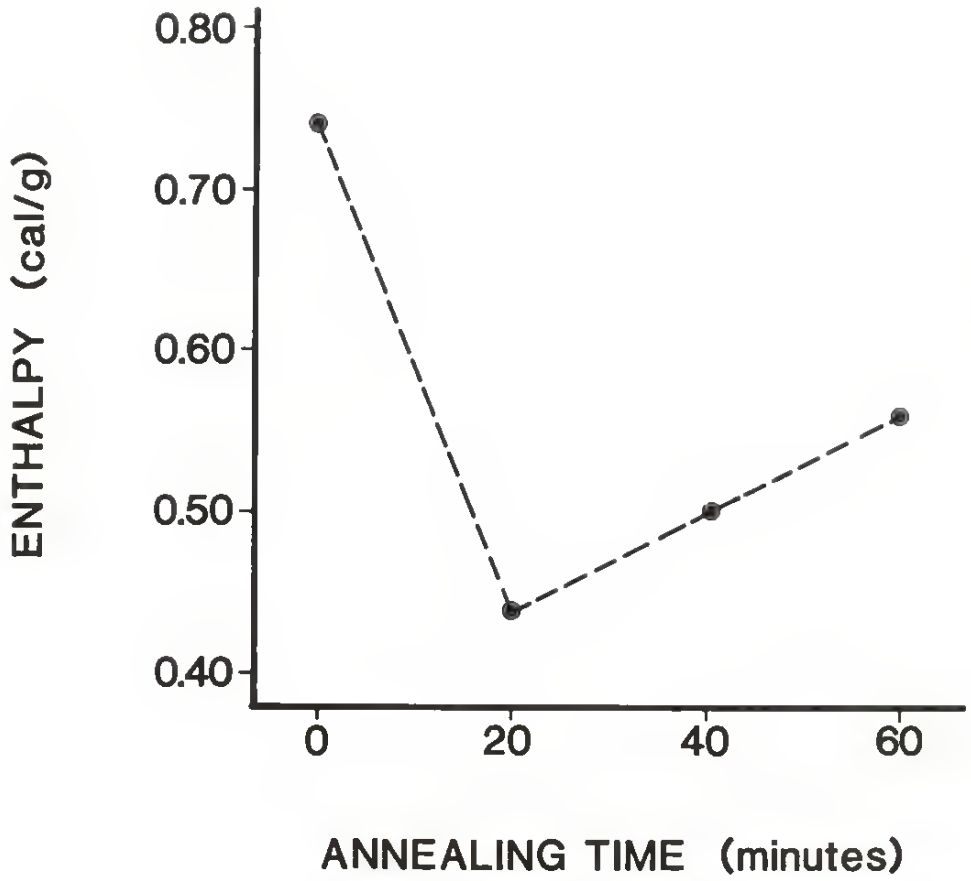
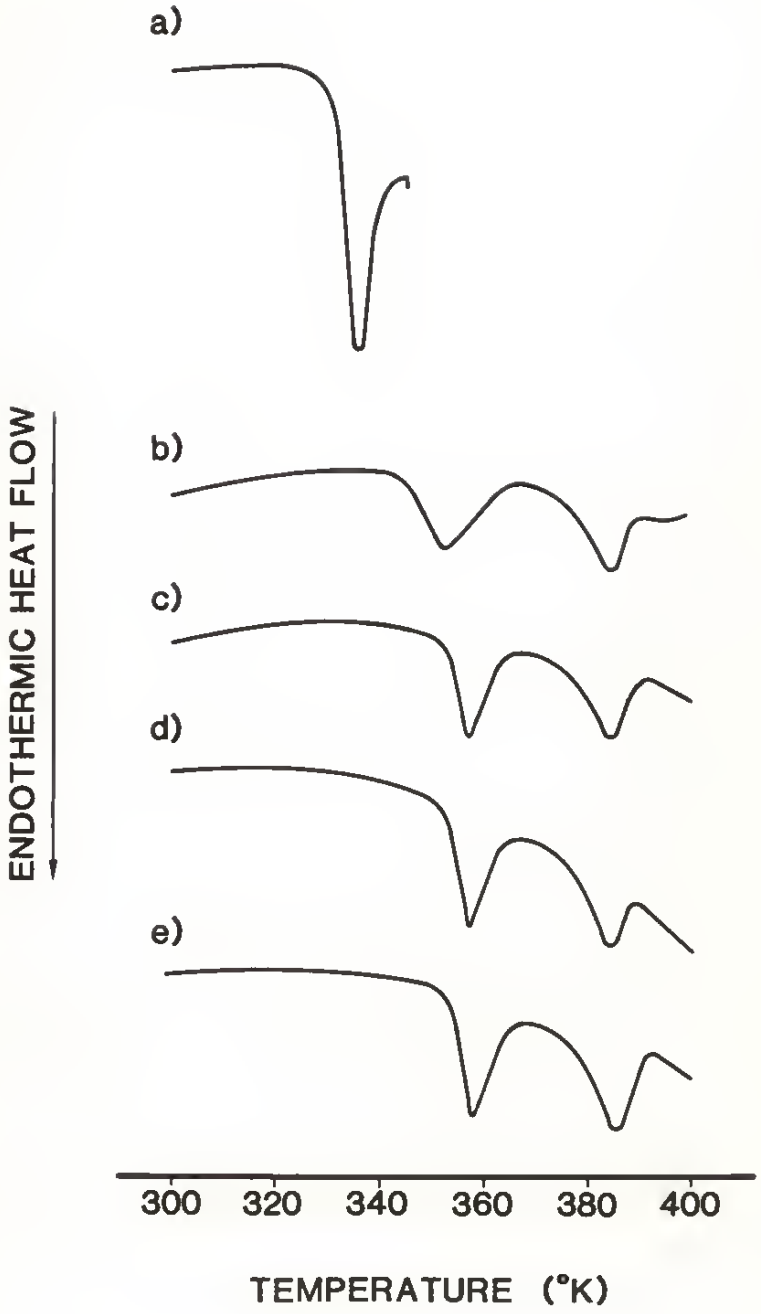

0.80
0.70
0.60
0.50
0.40
ENTHALPY (cal/g)
0
20
40
60
ANNEALING TIME (minutes)

Figure 9. DSC thermograms of 50% moisture wheat starch a) heated to 345°K and the heating program stopped, b) a rerun of starch heated to 345°K and immediately cooled to 280°K, c) a rerun of a sample heated to 345°K and annealed at that temperature for twenty minutes, then cooled to 280°K, d) same as in c) except for forty minutes of annealing at 345°K, and e) same as in c) except for sixty minutes of annealing at 345°K.


a)
b)
c)
d)
e)
ENDOTHERMIC HEAT FLOW
300
320
340
360
380
400
TEMPERATURE (°K)

CHAPTER 4

STARCH COMPRESSION

INTRODUCTION

The concept of a glass transition occurring in starch has been mentioned and described in the Literature Review and also in the chapter on differential scanning calorimetry. Maurice et al. (1983) proposed that starch exhibits this 2nd-order transition, characterized by a change in the heat capacity of the system and a slight increase in volume, which will transform the granules from rigid, glassy particles into flexible and rubbery entities. Thus, granules that have passed through a glass transition can be molded and compressed, quite unlike native granules which possess amazing bridging properties. If this is indeed the case, then an instrument that can apply a constant pressure to a starch sample that is then heated should be able to detect the change in malleability of the granules as they pass through the glass transition. Maurice and his colleagues used such an apparatus - a volume dilatometer attached to a thermomechanical analyzer - to record the volume change of a heated starch sample. The plot of volume change versus temperature for starch was interpreted as characteristic of a partially crystalline polymer that had undergone a glass transition.

The instrumentation used for this type of test is no doubt quite sensitive. In this method, the sample is allowed to expand as the granules become swollen during gelatinization. It would also seem possible to test for the occurrence of a glass

transition in starch by placing a sample under a constant force, then heating the starch and recording its resistance to the force as the temperature is increased. According to the hypothesis, if a glass transition occurs, a starch granule will yield under the force and be compressed and molded to conform to the shape of neighboring granules. An instrument that can perform such a test is an Instron tester. Lancaster (1971) used an Instron to investigate volume - pressure relationships during compression of corn grits and also the rheology of the material as it was extruded through a small die. While the Instron would be less sensitive to subtle changes in the granules, the decrease in resistance of the starch to the applied force, given a sufficient sample size and sufficient force, should be significant enough to be detected.

MATERIALS AND METHODS

The wheat starch used in this study was obtained from Midwest Solvents, Atchison, Kansas. An Instron Universal Testing Instrument, Model 1130, was used to compress the starch. A capillary extruder (Figures 10 and 11) was constructed by the Physics Machine Shop at Kansas State University and could be fitted with a small, single hole die or dead-end plug.

The following procedure was used to test a starch sample. Starch, hydrated to various moisture contents, 10%, 20%, 30%, 40%, and 50%, was loaded into the capillary extruder fitted with the solid plug. Small squares of paper towel were packed above and below the starch in the capillary to help distribute the force over the entire surface of the sample. A heating tape was

wrapped around the apparatus and a thermocouple inserted to monitor the temperature. The small extruder was mounted on the Instron in the manner shown in Figure 12. A force was applied to the starch and allowed to equilibrate - i.e., until a straight line was obtained on the force versus temperature readout of the chart recorder. Once this constant force was obtained, heat was supplied to the system through the heating tape at a rate of 4^{o}-8^{o}C per minute and the changes in the resistance of the starch to the constant force were recorded.

RESULTS AND DISCUSSION

A series of runs was obtained for all five moisture contents using a constant force of 16-18 kg-force (see the Appendix at the end of this chapter). The force versus temperature displays for all moisture levels possessed basically the same pattern - a slight initial increase in sample volume followed by a steady decrease as the capillary was heated (Figure 13). What did vary among moisture contents was the rate at which the sample volume decreased with increasing temperature - the higher moisture samples yielded to the force rather quickly whereas the drier starches exhibited a more gradual response to the heat and pressure. The higher moisture starches (40% and 50%) were transformed into a melted, semi-translucent rod, while the lower moisture contents retained their starchy - opaque nature (Figure 14). Although these relatively dry samples were pressed into a compact cylinder, they did not possess the same cohesive property characteristic of the 40% and 50% moisture treated starches and could be broken down into smaller pieces. The microscopic

differences in the treated starches can be easily seen in Figure 15. The higher moisture samples have obviously been gelatinized and, most likely, have passed through a glass transition. Since the glass transition temperature is dependent upon the amount of water in the starch, it is not entirely surprising that the low moisture sample appears unaffected. In addition, possible moisture loss from the starch during heating, the inability to obtain an actual temperature reading for the starch in the capillary, and the behavior of the packing material during compression and heating are some areas that need further investigation before these results can be fully interpreted.

None of the traces possessed a change in slope that would indicate the starch had undergone a glass transition. What was surprising was the immediate response of the system to the application of heat, such that at only a few degrees above room temperature, each sample apparently expanded against the applied force. Soon after heating began, this expansion would reverse, usually below 30°C, and the sample would begin to decrease in volume, with the rate of decrease depending upon the moisture content of the starch. At slightly over 100°C in all the samples tested, the rate of volume decrease experienced a change in slope and the sample decreased in volume more quickly (Figure 13). This change does not correspond to any specific event during the heating of starch.

None of the events recorded by the Instron could really be characterized as changes brought about by a glass transition. Although it is true that this instrument is not as sensitive as many that are used to measure volume change in solids, it should

still be possible, given the correct conditions, for the Instron to show that once the granules are heated past their glass transition temperature, they can be more easily compressed and conformed when placed under a force. The temperature at which this would occur will vary depending on the moisture content of the starch, since glass transitions are dependent upon the plasticizer (in this case water) content of a polymer (Maurice et al. 1983).

At first, it was thought that the initial increase in sample volume was due to a glass transition since an increase in the coefficient of volume expansion usually accompanies such a transition (Brydson 1972). This would have indicated that Tg is pressure dependent - that the transition could be forced to occur at a very low temperature if the starch is placed under great pressure. However, the idea that this volume increase was due to a glass transition has been discounted based on starch tested under a lower initial compressive force. These samples exhibited a larger increase in volume when heat was supplied to the capillary and this increase lasted for a longer period of time. For instance, when 850 g of force was applied and the sample heated, the volume increase moved the pen off scale and it did not return. At this time, no explanation is offered for this phenomenon. It is still quite possible that the glass transition is dependent upon pressure, but it may not be possible to test this concept under the original conditions of 16-18 kg-force. A smaller compressive force should be used since this is a tremendous amount of pressure applied to the small surface area of the sample.

CONCLUSION

In this experiment, an Instron tester was used to record the resistance of a heated starch sample to a constant compressive force. The profile obtained showed that with increasing temperature, starch granules become "softer" or more malleable, and become less resistant to an applied force. The rate of decrease in resistance to pressure was found to be dependent upon the moisture content of the starch - the higher moisture samples lost their rigidity over a shorter period of time. No event on the force versus temperature recording was attributable to a glass transition although it is quite possible that such a transition does occur. The inability to detect this event could be related to an excessively large amount of force used to compress the starch.

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APPENDIX

Kilograms-force (kg-f) is one of the derived force units associated with the Instron. In order to convert these derived units to more conventional force units, a few simple calculations must be performed. First, the pound-force (lb-f) unit in the American engineering system of units is defined as the product of a unit mass and the acceleration of gravity. Thus,

$$1 \text{ kg-f} = 1 \text{ kg} \times 9.8 \text{ m/s}^2;$$

and $$1 \text{ Newton (N)} = 1 \text{ kg m/s}^2,$$

so, $$1 \text{ kg-f} = 9.8 \text{ N}$$

The force applied by the Instron is then:

$$18 \text{ kg-f} \times 9.8 \text{ N/kg-f} = 176 \text{ N}$$

or $$176 \text{ N} \times 0.22481 \text{ lb-f/N} = 40 \text{ lb-f}$$

The bore diameter of the capillary extruder is 5.5 mm. The pressure upon the surface of the starch is then:

$$\frac{176 \text{ N}}{\pi(0.0055/2)^2 \text{ m}^2} = 7.41 \times 10^6 \text{ Pa}$$

or $$\frac{7.41 \times 10^6 \text{ Pa}}{1.01325 \times 10^5 \text{ Pa/atm}} = 73 \text{ atm}$$

or $$73 \text{ atm} \times 14.7 \text{ psi/atm} = 1070 \text{ psi}$$

Figure 10. Two views of the capillary extruder used to test the resistance of starch to a constant force as heat is applied to the system.

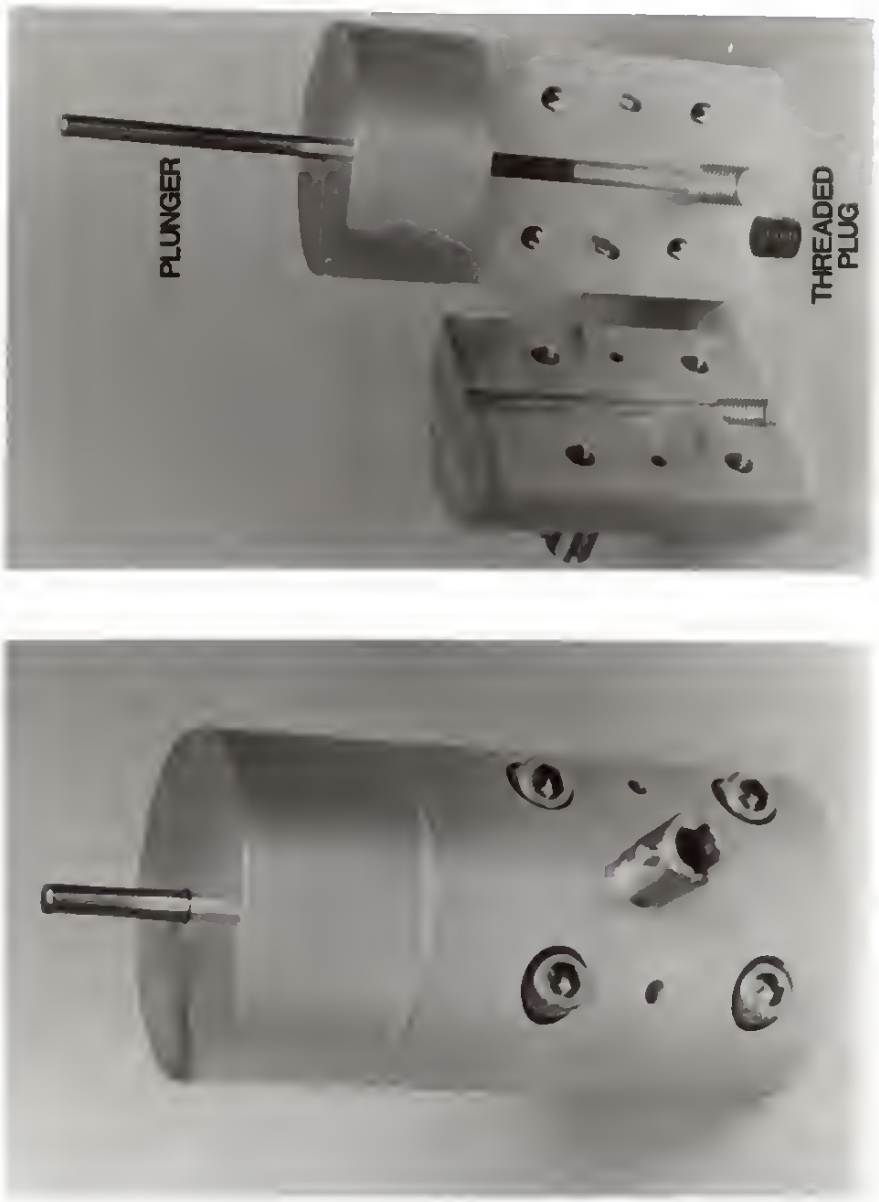
PLUNGER
THREADED PLUG

Figure 11. Schematic of the capillary extruder.

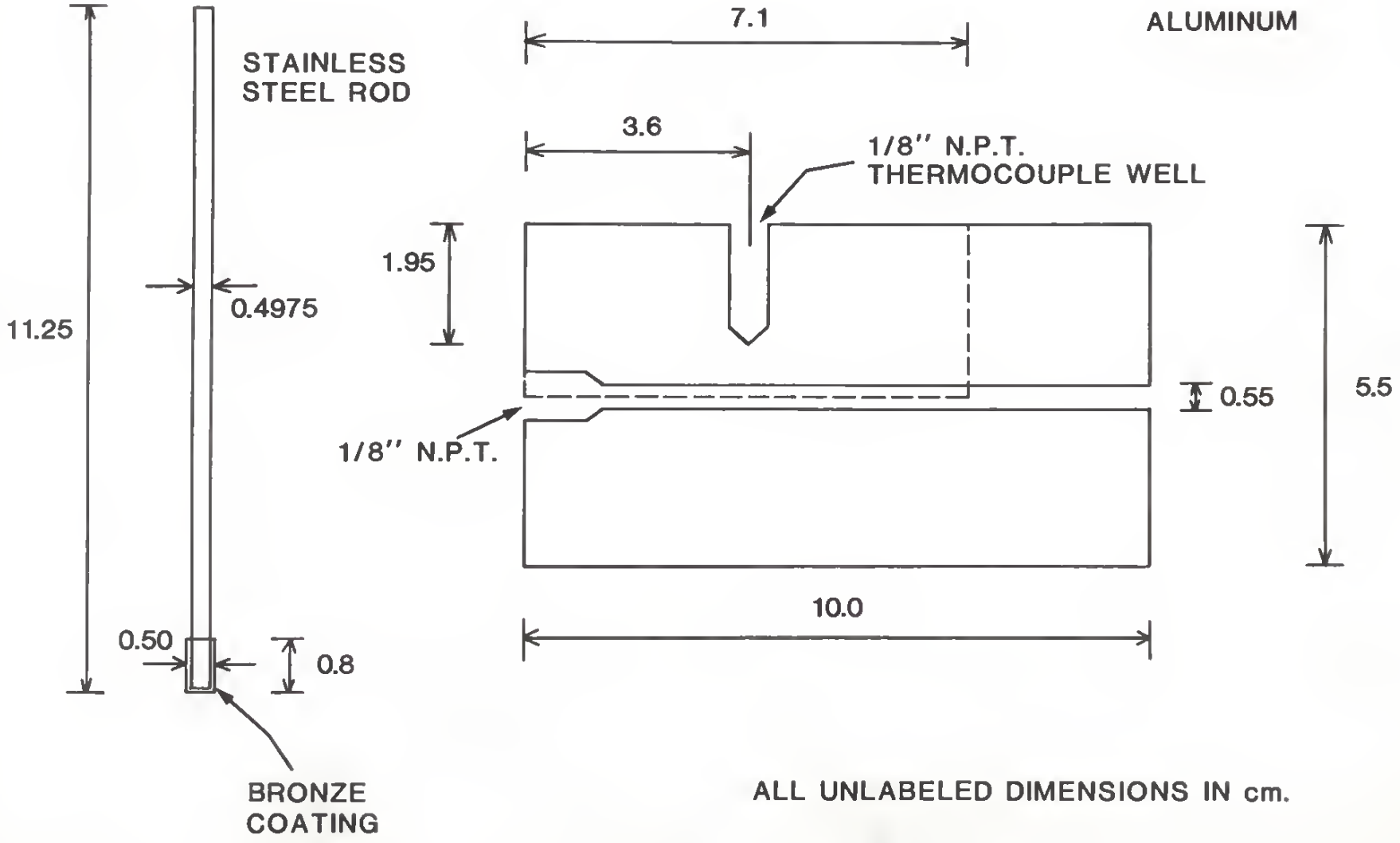
STAINLESS
STEEL ROD
11.25
0.4975
0.50
0.8
BRONZE
COATING
7.1
ALUMINUM
3.6
1/8" N.P.T.
THERMOCOUPLE WELL
1.95
0.55
5.5
1/8" N.P.T.
10.0
ALL UNLABELED DIMENSIONS IN cm.

Figure 12. Photograph showing the capillary extruder mounted on an Instron tester.

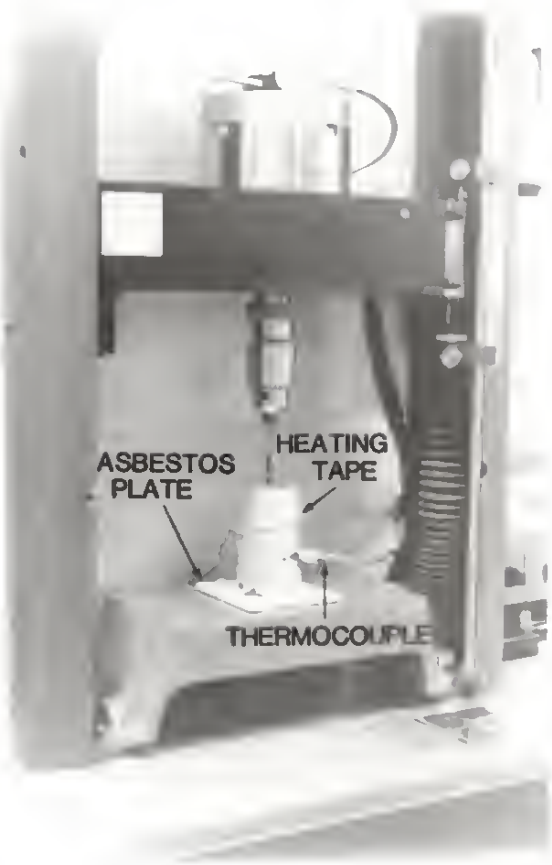
ASBESTOS
PLATE
HEATING
TAPE
THERMOCOUPLE

Figure 13. Graphical representation of the force versus temperature chart recordings obtained as starch, at five different moisture levels, was compressed then heated. The heating rate for all samples was between 4^{o}-8^{o}C per minute. The mark on each thermogram at 25^{o}C indicates where heating began.

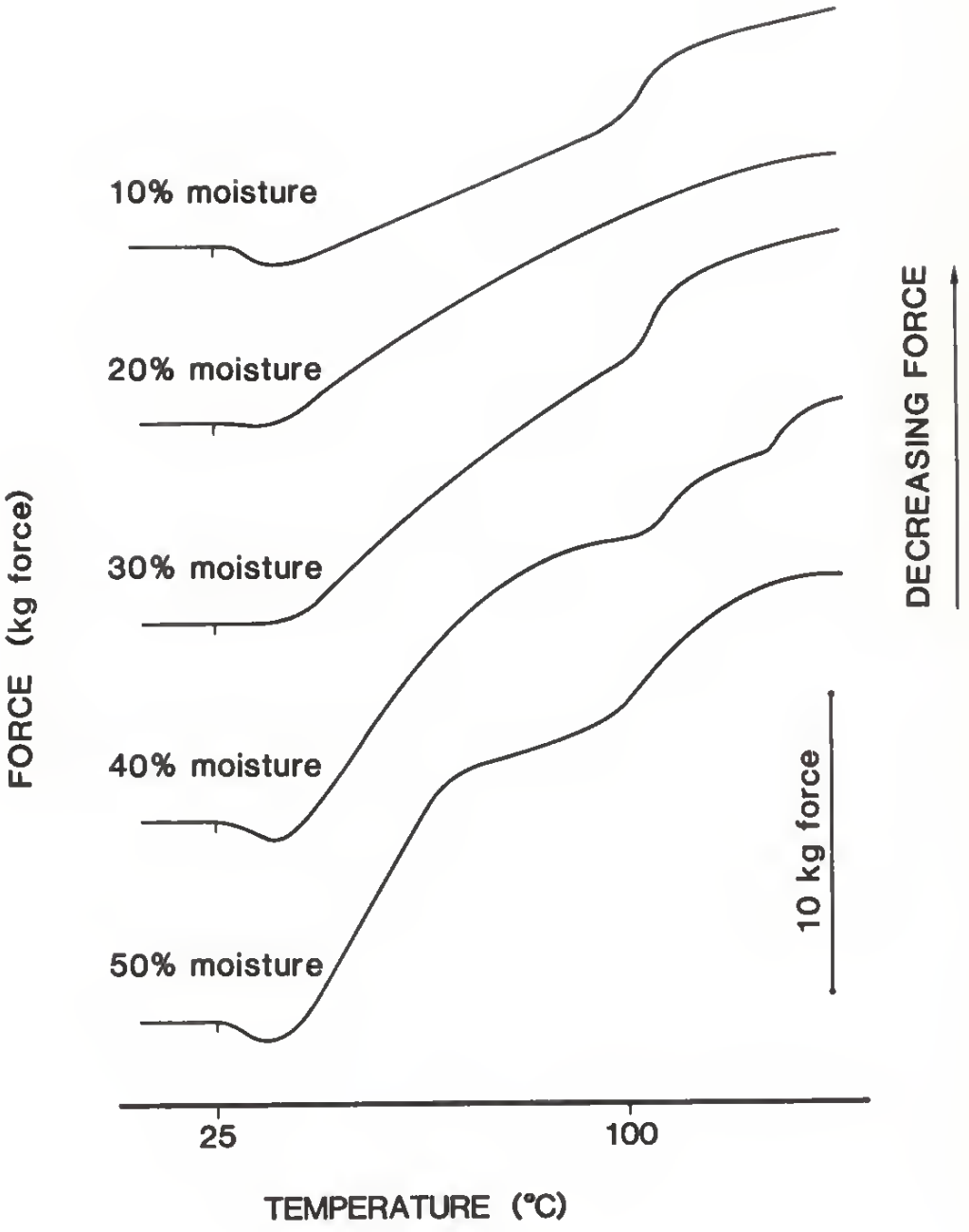
10% moisture
20% moisture
30% moisture
40% moisture
50% moisture
FORCE (kg force)
DECREASING FORCE
10 kg force
25
100
TEMPERATURE (°C)

Figure 14. Compressed starch plugs heated in the capillary extruder.

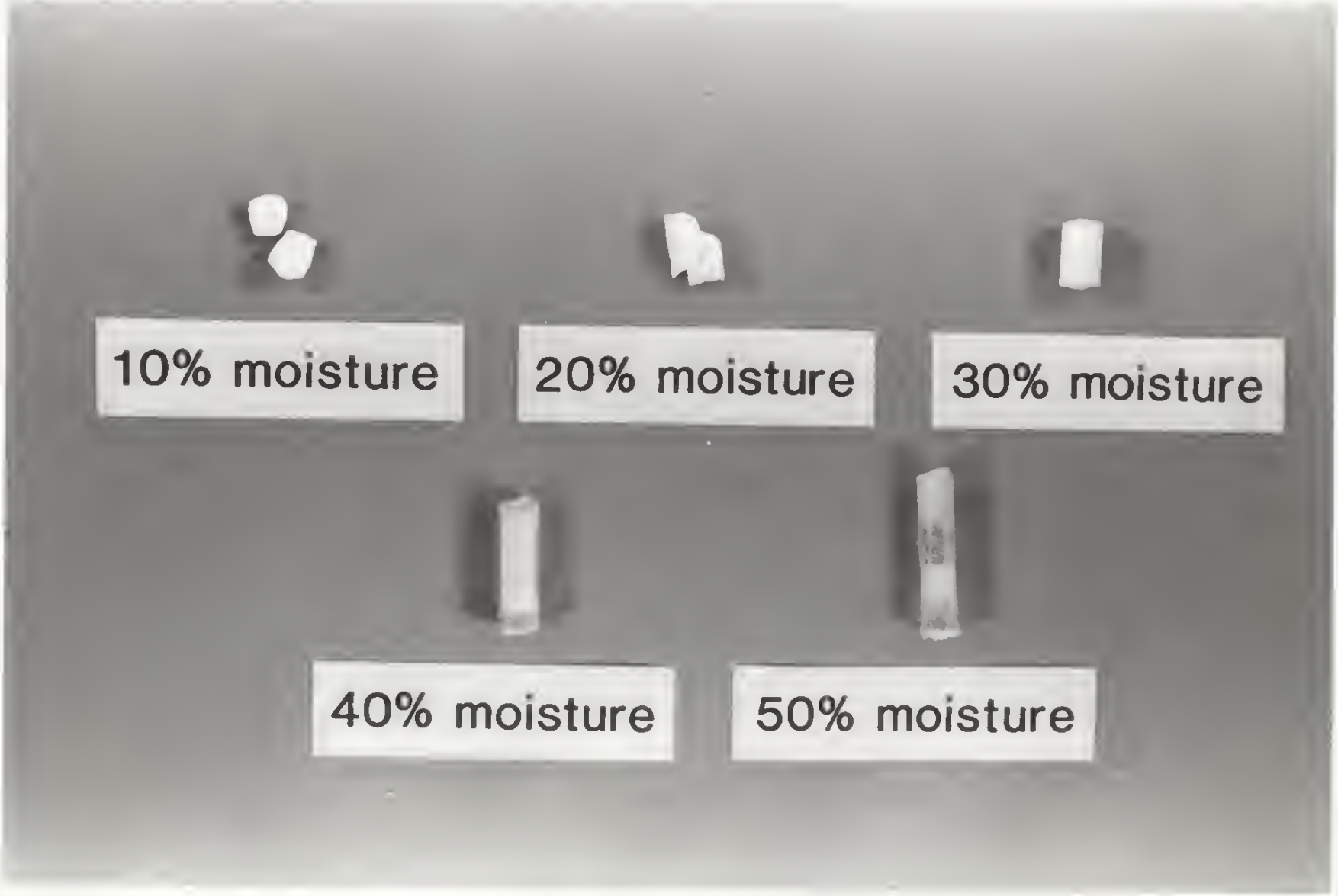
10% moisture
20% moisture
30% moisture
40% moisture
50% moisture

Figure 15. Scanning electron micrographs illustrating the effect of moisture content on wheat starch that was compressed and heated in the capillary extruder. The sample in a) was at 10% moisture; after 18 kg-force was applied to the starch, it was heated to 140°C. In b), the starch was at 50% moisture, compressed with 16 kg-force, and heated to 150°C. The bar in the lower right hand corner of each micrograph represents 10 μm.

(a)
(b)

CHAPTER 5

EXTRUSION

INTRODUCTION

High-temperature short-time (HTST) extrusion is one system where low moisture starch is thermally treated. Starchy or proteinaceous feed material is introduced into the extruder and is transported through the barrel by the rotating screw. Heat is produced from the frictional forces generated as the dough traverses the extruder. This heat, as well as any heat applied through the barrel jackets, will aid the screw in shearing, mixing, and working the material into a molten dough (Harper 1981). Due to the high pressures found within the extruder, the viscous extrudate will expand (or puff) as it exits the barrel through the die - the expansion being due to the rapid vaporization of super - heated water as it exits the die and encounters atmospheric pressure. With this type of process, many products such as collets, breakfast cereals, and flat breads are possible (Hauck 1979).

Most of what is known about food extrusion is derived from the field of plastics extrusion. This industry treats the material inside the barrel as a "homogeneous melt" of the plastic resin fed into the extruder. Starch and flour probably should not be analyzed exactly in this manner; allowances must be made for the differences between plastic polymers and starch or protein-based feed material. For example, if wheat flour is being extruded, starch, protein, lipid, and water are all

interacting within the extruder barrel creating a very complex system. Since HTST extrusion is a limited water system (usually below 25% moisture), the numerous studies performed on starch gelatinization in excess water will not apply in this situation. Extensive research has been performed upon the expanded products; much is known about the end results of the degradative process that takes place within a food extruder. However, discussions of specific mechanisms for this degradative process are less prevalent in the literature. Using a common sense approach, it can be suggested that the high temperatures "soften" (possibly by passing through a glass transition) and begin to melt the crystallites even at these low moisture contents. At this point, the order associated with starch granules is probably lost. With the loss of granular structure, the amylose and amylopectin molecules can now be dispersed into the viscous mass and exposed to further shearing and the high temperature and high pressure found in the metering section of the extruder. These molecules could possibly be cleaved into shorter-chained entities, and, as the molten material passes out the die, it is expanded as the super-heated water is vaporized.

Thus, one fundamental question facing the field of food extrusion is just what factors are responsible - and to what degree they are responsible - for the degradative process that occurs inside a food extruder. These factors are thought to include the parameters of temperature, pressure, shear, and the moisture content of the feed material. Another question that needs to be addressed concerns the formation of nucleating sites in the molten material. It has been suggested that starch

granules may be present in the "melt" (Faubion 1980), and that the hilum of these granules could act as nucleating sites for expansion similar to the system described for popcorn (Hoseney et al. 1983). This concept deserves further study since it has important ramifications for the current hypotheses of food extrusion.

MATERIALS AND METHODS

A laboratory scale extruder, a Brabender Model 2403, was used for this experiment. Wheat starch was obtained from Midwest Solvents, Atchison, Kansas. A die extension (Figures 16 and 17) was constructed by the Physics Machine Shop at Kansas State University which allowed a section of the molten material to be "captured" for examination before it had left the die and expanded.

The die extension was pre-heated with a heating tape to the temperature used for zone 3 (150^{o}C), and its temperature was monitored and maintained by the use of a surface thermocouple connected to a digital thermometer. The starch was tempered to approximately 18% moisture (w.b.) before it was run in the extruder. Screw speed was set at 100 RPM; the temperature controls were set at 0^{o}C (air-cooled) for zone 1, 100^{o}C for zone 2, and 150^{o}C for zone 3.

Capturing of the material was performed by closing the two ball valves on either side of the split - barrel section (see Figure 16) while the extruder was running and opening the release valve which allowed the extruder to be shut down in a normal fashion. As soon as extrusion had ceased, the die extender was

disassembled from the extruder and allowed to cool to room temperature before the unexpanded sample was removed (Figure 18). These samples were examined with an ETEC Autoscan Scanning Electron Microscope operating at a 10kV accelerating voltage.

RESULTS AND DISCUSSION

The main thrust of this portion of the research was to develop a method to investigate the effects of various extrusion parameters on the properties of extruded starch. It is obvious that cell size and cell distribution in an extruded rod will help to determine many of the properties of that rod, i.e., strength (resistance to a breaking force), bulk density, and expansion index. Hence, cell size and distribution could be used as a means of correlating the effects of individual extrusion parameters upon product quality. It was decided that one of the best approaches to study these extrudate properties would be to examine the material inside the extruder barrel for the nucleating sites responsible for the expansion of the product. Construction of the die extender provides a method for examining and characterizing the nucleating sites responsible for cell size and distribution in the final product.

Initially, the expanded product obtained with the die extender possessed large air cells as compared to the rods obtained with a normal 1/4" die cap - die adaptor assembly. This was attributed to a nonair-tight seal in the split - barrel section of the extender which allowed the molten material to experience a slight drop in pressure as it traversed the die extender. Consequently, some of the air cells present in the

material coalesced and formed larger air cells which resulted in increased cell size in the puffed product. Material that had not yet reached the split barrel portion of the die extender appeared very uniform - did not possess any visible air cells - whereas once the viscous mass encountered the first joint of the split barrel, large, visible air cells could be seen in the captured rod. This was remedied by coating the split barrel junctions with silicon caulk; a definite reduction in the number of visible air cells was seen in the captured section along with a return to more uniform air cells in the expanded rod. Upon cooling, the captured, unexpanded portion of the starchy material possessed a glassy, translucent appearance and was quite hard. When a force was applied perpendicular to the rod's major axis, it would shatter into small pieces. A visual inspection revealed that the rod consisted of a very homogeneous substance; it had the characteristics of a glassy, molded polymer with no traces of individual particles, i.e., starch granules.

As stated earlier, some of the samples collected in the early extrusion runs contained large air bubbles located primarily in the periphery of the captured rod. It was thought that these air cells were the cause of the large cell structure found in the extruded product. When the extender was caulked, the large cells disappeared and no air bubbles were visible with the naked eye in the unexpanded section (Figure 19). With the scanning electron microscope, however, numerous small air bubbles could be seen and they appeared to be more concentrated towards the outer edge of the rod (Figure 20). The finding that the distribution of air cells seems to be more dense in the outer

portions of the rod would explain why the large air cells found with an uncaulked die extender appeared to be located mainly in the periphery of the section. The nonuniform distribution of air cells would also discount the hypothesis that the hila of starch granules are the source for these air bubbles. Thus, these small air cells found with the SEM are no doubt the nucleating sites for the vaporization of super-heated water when the material exits the die. It was very evident here that the size of these air bubbles will have a tremendous effect upon cell size and distribution in the puffed product. Therefore, the die extender could be used to study the effect of extrusion parameters upon the size and distribution of nucleating sites entrained into the material inside the barrel; it is these nucleating sites which directly influence many of the properties of extruded products.

The Brabender extruder has a rather large screw length to screw diameter ratio which will impart a large amount of shear to any extruded substance. Shearing of the starch exposed to this combination of temperature and moisture could account for the thorough destruction of granular structure and the subsequent homogeniety of the feed material. The exact process by which the granules are broken down and the extent to which the starch molecules are disrupted is still not known. However, it is obvious that the die extender could be used to study these areas also.

CONCLUSION

The "capturing" apparatus constructed for this study allows material that has been treated in an extruder to be examined

before it has been expanded by exiting the die. It was noted that many very small air cells (not visible with the naked eye) exist in the rod and appear to be most heavily distributed in the periphery of the captured rod. These small air cells are the nucleating sites for vaporization of super-heated water as the molten starch exits the die and expands. The size and distribution of nucleating sites have a tremendous influence upon the physical properties of the expanded material. In turn, the size and distribution are dependent upon the extrusion parameters being used. Thus, the die extender can be a very valuable tool to help determine the effect of individual extrusion parameters upon the rheology of the material within the barrel and the resultant influence on the extruded product. No starch granules or any individual particles could be seen in a scanning electron micrograph of the unexpanded material. Also, evidence was found to discount the hypothesis that the hila of starch granules are the source of the nucleating sites for water vaporization, since the air cells were not distributed uniformly throughout the captured rod. In additon to investigating the size, distribution, and formation of nucleating sites during extrusion, the die extender could also be used to study granular disruption and molecular changes that occur during extrusion.

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Figure 16. Photograph of the die extender constructed to collect starch samples before the molten material exits the extruder die.

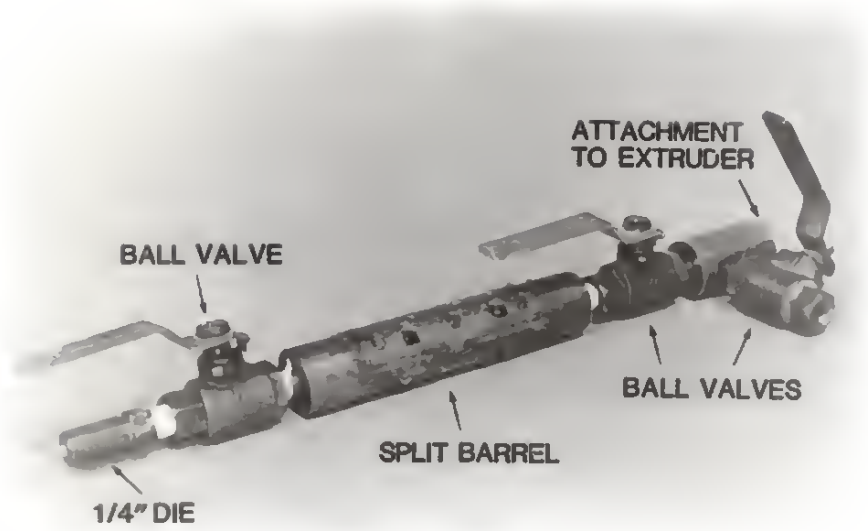
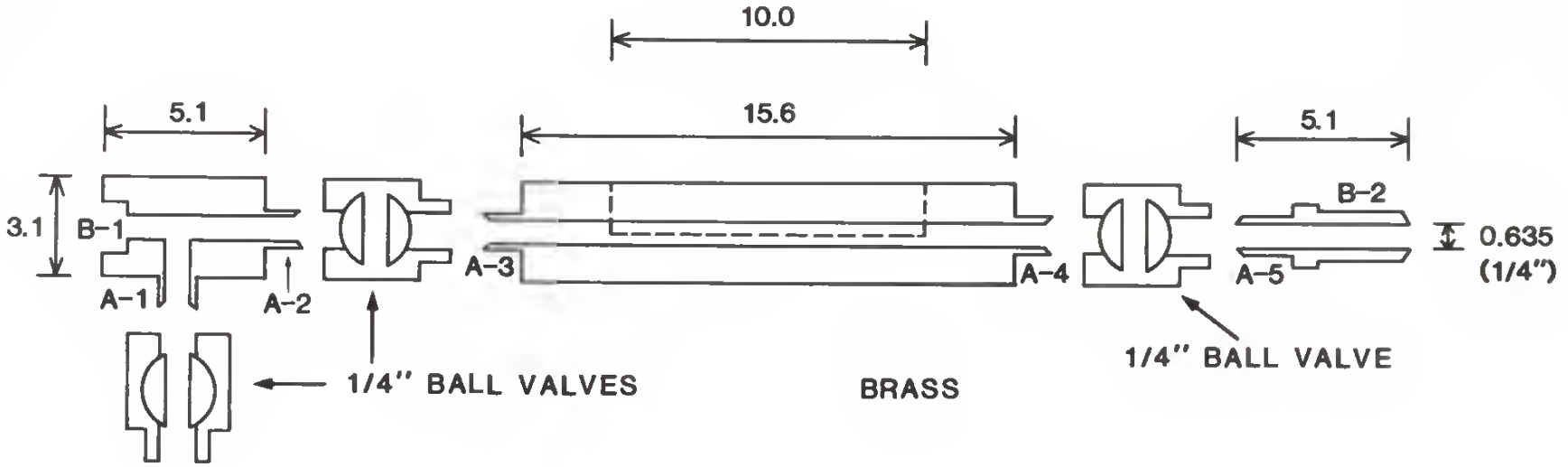

ATTACHMENT
TO EXTRUDER
BALL VALVE
BALL VALVES
SPLIT BARREL
1/4" DIE

Figure 17. Schematic of the die extender.



ALL UNLABELED DIMENSIONS IN cm.

A-1: 1/4" N.P.T., 1.6 cm

A-2: 1/4" N.P.T., 1.7 cm

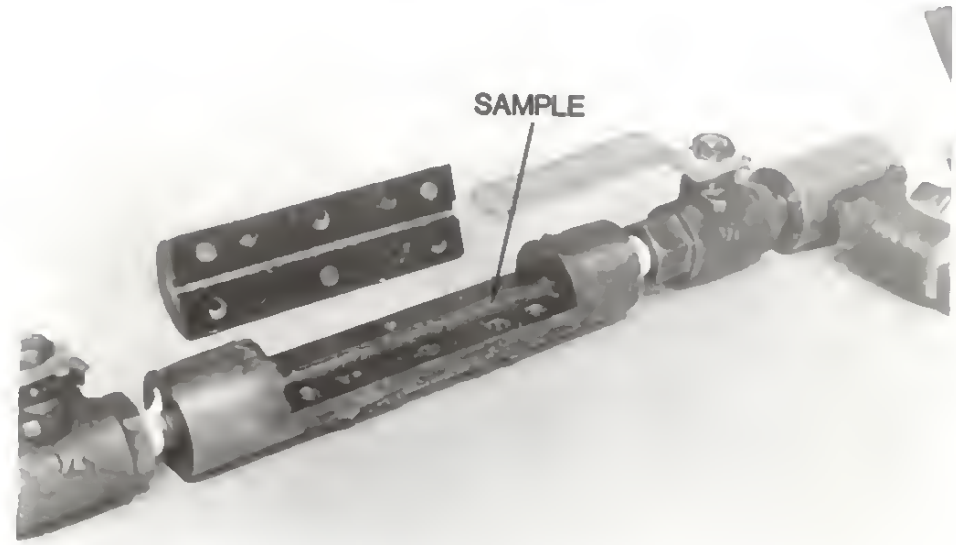
A-3: 1/4" N.P.T., 1.6 cm

A-4: 1/4" N.P.T., 1.9 cm

A-5: 1/4" N.P.T., 1.6 cm

B-1 and B-2: 5/8" 18

Figure 18. Photographs of an unexpanded starch sample before and after removal from the split-barrel section of the die extender.


SAMPLE

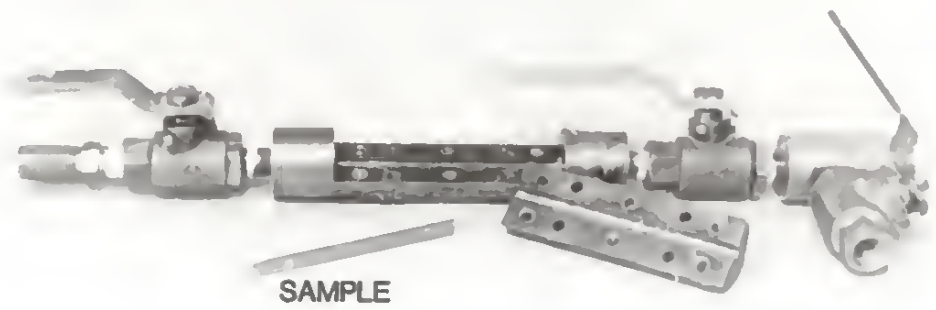
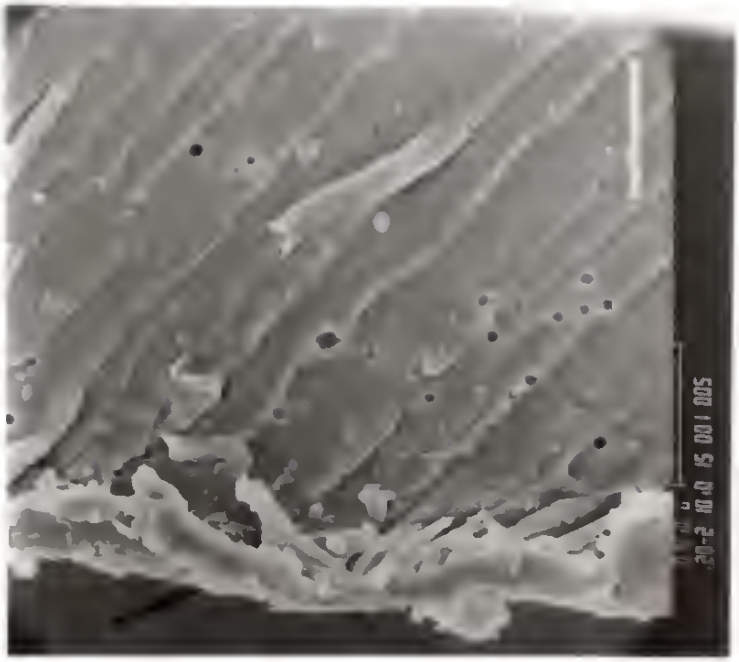
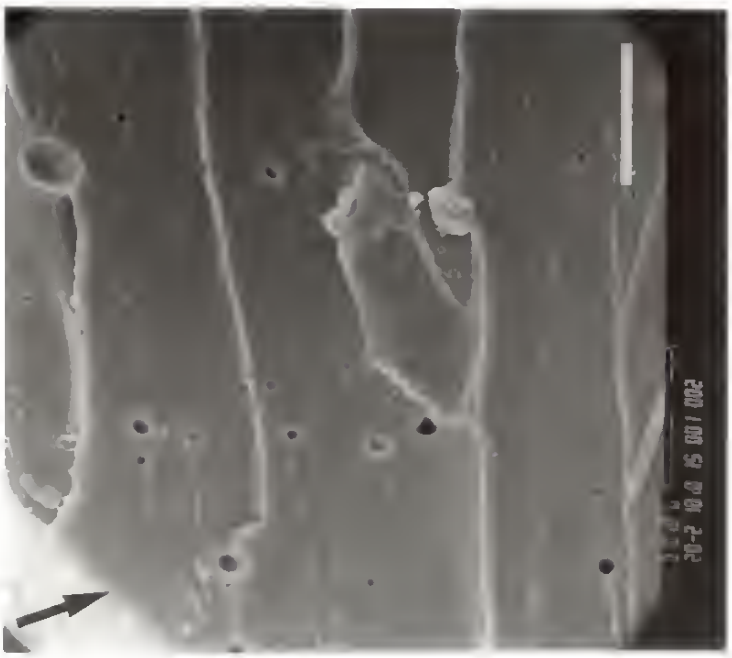

SAMPLE

Figure 19. Scanning electron micrographs showing a) the very large air cells present in an unexpanded starch rod obtained with an uncaulked die extender and b) a starch rod with no visible air cells obtained after sealing the extender with silicon caulk. In b), the circular depressions in the lower right and upper left portions of the sample are grooves that had been etched along the entire length of the rod. The bar in the lower right hand corner of each micrograph represents 500 μm.

(b)

Figure 20. Scanning electron micrographs showing the distribution of small air cells towards the outer edge of the captured rods. The arrows indicate the edge of the samples. The bar in the lower right hand corner of each micrograph represents 10 μm.





CHAPTER 6

HEAT TREATMENT

One problem encountered during the course of this work which deserves comment concerns heat treatment of low moisture (ca 30% moisture or below) starch or flour. When heating these samples in a sealed container, the amount of air space above the material can affect the treatment conditions. As heating begins, the vapor pressure of water in the container will rise in accordance with the increasing temperature of the system. Water will be driven out of the starch or flour to accomodate the increased vapor pressure, resulting in lower moisture contents when the treatment temperature is reached. Fortunately, this does not reduce the moisture content a significant amount in most instances; however, it is imperative to place the sample in a sealed container, or all the water in the sample will be driven off. Determining moisture contents after the treatment will not give an indication of what occurred during heating, since any moisture driven out of the sample will simply be reabsorbed by the starch or flour when the vessel is cooled. Because the water vapor pressure will be determined by the treatment temperature, the only variable that can be controlled to diminish the effect is to create a sample volume to container volume ratio of close to one by either increasing the size of the sample or by decreasing the size of the containment vessel. In addition to minimizing the volume of air in the treatment container, it is advantageous to heat thin sections of the starch or flour. Thin,

flat containers (such as moisture dishes for small samples) will prevent large temperature gradients in the sample which can cause a problem at moisture contents from approximately 30% moisture up to the point at which water will be in excess. When heating bulky samples in this moisture range, those portions of the material closest to the source of heat (next to the walls of the container) may gelatinize and withdraw water from the rest of the sample, creating a distinct moisture gradient and nonhomogeneity of the treated sample (Ghiasi et al. 1982).

To help illustrate the effect that void space can have on a heated sample, a few basic calculations have been performed. These are hypothetical situations in treating starch at various conditions of temperature, sample size, container size, and moisture content. These results are, at best, only approximate, but they should provide a general understanding of how void volume affects the moisture content of starch undergoing heat treatment.

Table 5 lists the treatment variables. A sample calculation for a 50 g starch sample at 10% moisture heated to 100°C in a pint sample jar will be used as an example. The equations referred to in this example can be found in the Appendix. Because the sample is 10% water, 90%, or (0.90)(50 g) = 45 g is dry starch. The density of starch is assumed to be 1.55 g/cm^3 and for water 1.00 g/cm^3 (Donovan 1979). Thus, the volume occupied by the sample in the container, found in equation 1, is 34 cm^3. It follows, then, that the void volume is the capacity of the container, 482 cm^3, minus the sample volume, or 448 cm^3 (equation 2). The remaining volumes are provided in Table 6. It

is now possible to calculate the amount of water driven out of the sample by using the Ideal Gas Law. Appropriate values from Tables 5 and 6 are substituted into equation 3, and equation 4 converts moles of water lost into grams of water lost. Finally, the new moisture content of the sample at the specific treatment conditions can be found (equation 5). Table 7 lists the moisture contents calculated for all the treatment combinations.

It can be seen that with reasonable precautions, any large, adverse effects can be avoided. Careful consideration in choosing a containment vessel for heat treating starch or flour samples can prevent greatly altering the moisture content as would be found if 50 g of a sample were heated in a quart - sized jar to 150°C. According to Table 7, although this starch was originally at a moisture content of 20%, it would lose enough water to be at less than 12% (11.9 %) during the treatment, and starch originally at 10% moisture would be practically dry at a water content of less than 1% (0.9%). Conversely, if a larger sample size was heated in a smaller container, this effect would be greatly diminished. For instance, treating 100 g of 20% moisture starch in a pint - sized jar at 150°C would decrease the moisture content less than 1%, or down to 19.2%. In order to present a more practical example, similar calculations were performed with starch samples heated in Perkin-Elmer stainless steel sample pans. In these pans, a 20% moisture, 5 mg starch sample would be 18.5% moisture at 120°C and a 10% moisture sample would be reduced to 8.3% at the same temperature. Although the starch loses water during the scan, the amount of void volume in the stainless steel pans is small enough such that the decrease

in moisture content should not significantly alter the thermogram. Finally, when publishing or reporting data where low moisture starch or flour has been heat treated, information on the size of the containment vessel and size of the sample should also be provided.

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Table 5. Listing of the Hypothetical Treatment Variables.

Treatment Temperatures (^{o}K, ^{o}C)	Corresponding Water Vapor Pressure (bars)
373 , 100	1.01325
393 , 120	2
413 , 140	3.6
423 , 150	4.8

Container Volumes: Pint jar: 482 cm^3

Quart jar: 1905 cm^3

Hypothetical Starch Samples:

50 g of starch at 10% moisture

50 g of starch at 20% moisture

100 g of starch at 10% moisture

100 g of starch at 20% moisture

Table 6. Volumes Calculated Using Equations 1 and 2 From the Appendix.

	Starch Sample			
	50 g/ 10%	50 g/ 20%	100 g/ 10%	100 g/ 20%
Starch Volume (cm^3)	34	31	68	62
Void Volume in Jars (cm^3) pint	448	451	414	420
Void Volume in Jars (cm^3) quart	1871	1874	1837	1843

Table 7. Starch Moisture Contents During the Hypothetical Heat Treatment.

	Starch Sample							
	50 g/ 10%		50 g/ 20%		100 g/ 10%		100 g/ 20%	
Treatment Temperature	Pint Jar	Quart Jar	Pint Jar	Quart Jar	Pint Jar	Quart Jar	Pint Jar	Quart Jar
100°C	9.5%	8.0%	19.6%	18.2%	9.8%	9.0%	19.8%	19.1%
120°C	9.1%	6.1%	19.2%	16.6%	9.6%	8.1%	19.6%	18.3%
140°C	8.4%	3.2%	18.6%	13.9%	9.3%	6.8%	19.4%	17.1%
150°C	8.0%	0.9%	18.2%	11.9%	9.1%	5.7%	19.2%	16.2%

Appendix

The Equations Used in Chapter 6.

Equation 1:

$$\frac{45 \text{ g starch}}{1.55 \text{ g starch / cm}^3} + \frac{5 \text{ g } H_2O}{1 \text{ g } H_2O \text{ / cm}^3} = 34 \text{ cm}^3$$

Equation 2:

$$482 \text{ cm}^3 - 34 \text{ cm}^3 = 448 \text{ cm}^3$$

Ideal Gas Law:

$P V = n R T$, where

P = Pressure
V = Volume
n = Moles of Gas
R = Gas Constant
T = Absolute Temperature

Rearranging,

$$\text{mols } H_2O \text{ driven out of the sample} = \frac{(\text{Vapor Pressure})(\text{Void Volume})}{(\text{Gas Constant})(\text{Temperature})}$$

For the 50 g, 10% moisture starch sample in the pint jar heated to 100°C,

Equation 3:

$$\frac{(1.01325 \text{ bars})(448 \text{ cm}^3)}{(83.14 \text{ bar}\cdot\text{cm}^3/\text{mol}\cdot{}^\circ\text{K})(373{}^\circ\text{K})} = 0.0146 \text{ mols } H_2O$$

Equation 4:

$$0.0146 \text{ mols} \times 18 \text{ g/mol } H_2O = 0.26 \text{ g } H_2O$$

0.26 g H_2O driven out of the sample

Equation 5:

$$\frac{5 \text{ g } H_2O - 0.26 \text{ g } H_2O \text{ lost}}{50 \text{ g sample} - 0.26 \text{ g } H_2O \text{ lost}} = 9.5\%$$

9.5% new moisture content of the starch sample at 100°C

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GELATINIZATION OF LOW MOISTURE WHEAT STARCH

by

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B. S., Kansas State University, 1982

AN ABSTRACT OF A MASTER'S THESIS

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requirements for the degree

MASTER OF SCIENCE

Department of Grain Science and Industry

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1985

ABSTRACT

The gelatinization phenomenon of wheat starch was studied with an emphasis placed on elucidating the events occurring at the molecular level during this transition. Upon review, the current hypotheses for gelatinization were not entirely satisfactory and a new hypothesis for the gelatinization mechanism was presented. This proposed mechanism suggests that the melting of starch crystallites in an excess of water begins at the hilum and proceeds rapidly towards the outer edges of the granule due to swelling in the melted regions imparting stress on nearby crystallites.

In this project, gelatinization was not approached as a single endothermic transition, but was studied as a combination of endothermic events (such as melting of the crystallites and swelling of the granule) and exothermic events (such as hydration of the exposed starch chains and formation of the amylose-lipid complex). For example, the hypothesis that gelatinization enthalpy decreases as the amount of water in a sample decreases was investigated with an adiabatic calorimeter to test whether one of the endothermic components was not being fully expressed due to less available water. When excess water was added to wheat starch which had been heat treated at low moisture, no response, indicative of a residual effect, was seen.

A differential scanning calorimeter was used to investigate whether starch exhibits a glass transition. It has been suggested that this transition occurs as gelatinization begins; however, it was found that changes due to a glass transition are noticed well before the onset of gelatinization. Annealing of

starch crystallites did not appear to increase gelatinization enthalpy. At a 1:1 water-to-starch ratio, gelatinization enthalpy was dependent upon the length of sample equilibration prior to heating. This was explained as a hydration phenomenon - that with less than excess water, more time is required for the water to migrate and hydrate accessible starch chains. If heated soon after the addition of water, these accessible but 'dry' chains will be hydrated upon gelatinization, an exothermic event. If these chains are fully hydrated prior to heating, more of the endothermic contribution to gelatinization will be expressed, giving a larger enthalpy value.

The glass transition was also studied using an Instron to compress starch as it was heated. No specific event could be attributed to a glass transition, but, under a large, constant force, the starch was easily compressed after the application of heat at temperatures well below gelatinization. This may indicate that a glass transition did occur, and that the transition may be pressure dependent.

A device was constructed to collect extruded starch samples as they would exist in the die prior to puffing. This material was found to be very homogeneous - devoid of any discrete particles. Many small air cells were distributed towards the outer edges of the samples; these cells are thought to be the nucleating sites for water vaporization as the starch exits the die and expands.